ICES WKFATHOM2 REPORT 2018

ECOSYSTEM OBSERVATION STEERING GROUP

ICES CM 2018/EOSG:22

REF ACOM AND SCICOM

Report of the Workshop on egg staging, fecundity, and atresia in horse mackerel and mackerel (WKFATHOM2)

8-12 October and 19-23 November

Bremerhaven, Germany and IJmuiden, Netherlands

ICES
International Council for the Exploration of the Sea

CIEM
Conseil International pour l'Exploration de la Mer

International Council for the Exploration of the Sea
Conseil International pour l'Exploration de la Mer

H. C. Andersens Boulevard 44–46
DK-1553 Copenhagen V
Denmark
Telephone (+45) 33 38 67 00
Telefax (+45) 33 93 42 15
www.ices.dk
info@ices.dk

Recommended format for purposes of citation:

ICES. 2018. Report of the Workshop on egg staging, fecundity, and atresia in horse mackerel and mackerel (WKFATHOM2). 8-12 October and 19-23 November. Bremerhaven, Germany and IJmuiden, Netherlands. ICES CM 2018/EOSG:22. 74pp.
https://doi.org/10.17895/ices.pub.8176

Contents

Executive summary

The Workshop on egg staging, fecundity, and atresia in horse mackerel and mackerel (WKFATHOM) met twice in 2018. The first meeting was from 8 to 12 October in Bremerhaven, Germany, to calibrate egg sorting, staging and identification. The second meeting was from 19 until 23 November in IJmuiden, The Netherlands, to calibrate fecundity and atresia estimation and to standardize analysis for the DEPM method.

The 'spray technique' for the removal of fish eggs from preserved plankton samples was again tested and shown to inexperienced participants. The technique was also evaluated for its proposed suitability to separate hake eggs from other eggs in the samples, because hake eggs appear to remain buoyant with the other plankton and do not sink.

The majority of the time at the workshop was spent identifying and staging mackerel, horse mackerel and similar eggs. The results promoted discussion and highlighted specific problem areas. These discussions led to the further development of standard protocols, and enhancements to the species and stage descriptions. The results were very reassuring and with respect to the staging even better than those obtained at the 2015 workshop. For the experts there was an underestimate of stage 1 mackerel eggs (stages 1a and 1b combined) during the first round of analysis (-2%) and a 1% overestimation during the second round. The results for stage 1 horse mackerel eggs were 1% and 2% overestimation for round 1 and 2, respectively, for the experts. This is particularly reassuring as it is this stage on which the egg production estimates are based upon.

The screening, fecundity and atresia calibration proved beneficial to all participants. Particularly when % of non-agreement in exercises is high. For screening, clarification in the differentiation between the hydration and egg stages was necessary as well as classification of spent ovaries and massive atresia. For atresia, estimation problems occurred basically when the methodological routine was not correctly applied. After discussions, the manual has been improved. There was agreement on identification of vitellogenic and early alpha atretic oocytes. A few key features were agreed to define the transition of POF stages, but POF staging remains difficult. Further POF Stagging ring test among participants is required.

As the mackerel and horse mackerel egg surveys are carried out once every three years, these workshops are a refresher for expert survey participants and a first acquaintance for new participants in the sample analyses. It should however be realized that a one week workshop for either egg staging or fecundity analysis is not sufficient to train new participants. Institutes should allow newcomers to be thoroughly trained but also encourage more experienced participants to brush up on their knowledge properly before the survey.

1 Opening of the meeting

The Working Group on Egg staging, Fecundity and Atresia in Horse mackerel and Mackerel (WKFATHOM) met 08 - 12 October 2018 in Bremerhaven, Germany and 19 - 23 November 2018 in IJmuiden, The Netherlands. 24 participants from 10 countries (representing 11 different institutes) participated in the October meeting. 18 participants from 7 countries (representing 8 different institutes) participated in the November meeting. The participant lists can be found in Annex 1.

1.1 Background

In preparation for the 2019 international ICES coordinated mackerel (*Scomber scombrus*) and horse mackerel (*Trachurus trachurus*) egg survey, two workshops were held to standardize and calibrate the identification and staging of eggs and the estimation of fecundity.

The first workshop was held at TI-SF, Bremerhaven, Germany, for the majority of plankton analysts who will be involved in the 2019 survey. The aims of the workshop were to standardize procedures and produce definitive criteria for the identification and staging of mackerel and horse mackerel eggs. The workshop also investigated the reasons for individual differences in the identification and staging of mackerel and horse mackerel eggs and attempted to harmonize these. Evaluation of the use of the 'spray' technique, for removing fish eggs from plankton samples but also to separate hake eggs from other eggs, was carried out.

To enable the calculation of the numbers of spawning female fish in a stock by the Annual Egg Production Method (AEPM; Lockwood *et al.,* 1981, Armstrong *et al.,* 2001) or Daily Egg Production Method (DEPM; Lasker, 1985) it is essential to correctly identify (both in terms of species and age) the number of freshly spawned eggs, *i.e.* the eggs in development stages IA and IB, and to distinguish these from eggs in later stages of development but also from other species of the same stages. It is therefore vital that the analysts involved with sorting, identification and staging of mackerel and horse mackerel eggs from the triennial egg surveys are able to accurately identify and stage the eggs of each of the target species (ICES, 2015a). These workshops (WKFATHOM) were designed to bring the analysts together to develop consistent criteria for the identification and staging of the eggs, and to discuss how to overcome the practical problems encountered whilst doing so. Previous workshops (ICES, 2001; 2004; 2006; 2009; 2012; 2015b) have developed a comprehensive set of criteria for both mackerel and horse mackerel egg identification and staging. These criteria were reviewed during the 2018 workshop. With the exception of ling, no additions or changes were considered necessary for the identification criteria of both egg stage and species. For ling eggs, only a remark on the ambient temperatures where these eggs are most likely to occure was added. In addition, inexperienced analysts were involved for the first time, and it was critical that they became fully aware of the procedures and criteria in advance of the 2019 surveys in the Northeast Atlantic as well as in the 2020 surveys in the North Sea.

In addition to the correct identification of spawned eggs it is vital for egg production methods (EPM) to have a good estimation of potential fecundity, batch fecundity, atresia and spawning fraction in order to estimate Spawning-stock biomass (SSB). In order to calibrate estimations of fecundity and atresia a second workshop took place at Wageningen Marine Research, The Netherlands. Methods and criteria developed in previous workshops (ICES, 2006, 2009, 2012, 2015b) were expanded and further developed

during this workshop. Also inexperienced analysts were taught how to correctly identify vitellogenic and atretic oocytes and how to estimate fecundity and atresia.

1.2 Terms of Reference

The terms of reference for the meetings were:

a) Carry out comparative plankton sorting trials on typical survey samples. This should follow the pattern of trial – analysis – retrial – identification of problem areas;

b) Carry out a comparative egg staging trial for mackerel and horse mackerel eggs following the pattern used in the 2009 egg staging workshop;

c) Update a set of standard pictures and descriptions for species identification and egg staging;

d) Review available documentation on identifying eggs to species and define standard protocols;

e) Carry out inter-calibration work on fecundity and atresia determination and POFs staging;

f) Update a set of standard pictures for both oocytes and POFs stages;

g) Harmonize the analysis and interpretation of fecundity and atresia samples;

h) Review the methodology in use and available documentation on fecundity determination in order to redefine the standard protocols.

2 Adoption of the agenda

The agendas addressed all ToRs and were adopted without changes. The agendas can be found in Annex 2.

3 Materials and methods

3.1 Egg sorting trials (ToR a)

As a result of the egg sorting trials conducted during the previous workshops, most participating institutes are now using the 'spray technique' for routinely removing fish eggs from plankton samples (Eltink, 2007). Ulleweit et al. (2017) showed in a working document presented at the WGMEGS meeting in 2017 that hake (*Merluccius merluccius*) and also pearside (*Maurolicus muelleri*) eggs remain afloat among the rest of the plankton and cannot be removed by this technique. However, the spray technique could, thus, be used to separate hake eggs from mackerel and horse mackerel eggs. The spray method is described in detail in Eltink (2007) and the Manual for the mackerel and horse mackerel egg survey (ICES, 2019)

In an attempt to standardize and teach inexperienced participants the 'spray technique' two plankton samples (typical plankton from the 2016 survey) were prepared, each containing a known number of fish eggs. All participants, in groups of two, were asked to undertake the following procedure twice during the workshop to remove and count the eggs from the prepared samples.

The spray sorting exercise consisted of two rounds of two or three people per team. The spraying was repeated three times in each round. The numbers of eggs removed after each spraying and those eggs remaining in the plankton were counted using a binocular microscope.

The sample was then fully sorted using a binocular microscope, to remove any remaining eggs from the plankton. The eggs in the plankton sample were a mix of mackerel and hake eggs. The numbers of eggs removed after each spraying and those eggs remaining in the plankton were counted, and the results are presented in Table 4.1.1.

In addition, because hake eggs (*Merluccius merluccius*) are morphologically similar to those of mackerel and horse mackerel the Surface Adhesion Test (SAT) was applied in order to discriminate hake from other sorted fish eggs. The SAT procedure is fully described in Porebski (1975) and Coombs (1994).

During the first round, the SAT was performed after each spraying. However, due to time constraints, during the second round some the SAT was only applied at the end of the full sorting exercise by some participants.

3.2 Egg staging (ToR b, c and d)

3.2.1 Egg staging trials

A total of 540 mackerel, horse mackerel and hake (*Merluccius merluccius*) eggs as well as other species, which can be found in egg survey samples, were placed in 20 small, Perspex trays. Each tray contained 25 small wells but only the first 15 wells for the first round and 12 well for the second round were used to hold one egg each. Each tray was numbered and placed on the stage of a stereo-zoom microscope. The rows and columns of each tray were labelled so that the position of each individual egg could be identified. During the first round 300 eggs were staged by participants, while the second round the number of eggs was decreased to 240. The number of validated eggs was low for all of the species, in particular for horse mackerel, where also the total number of eggs, validated and unvalidated, was low. Unvalidated eggs were taken from the 2013, 2016 Atlantic and 2017 North Sea mackerel egg surveys. Some of the validated eggs also had known stages. The eggs were mainly those of mackerel and horse mackerel with a few hake eggs, which are morphologically similar to those of the two target

species. It was hoped that these definitive eggs, of known parentage, would allow participants' species identification to be judged more consistently. The eggs were selected at random with the intention of providing the full range of egg stages, but with greater emphasis on stage 1 eggs on which the estimates of SSB are based. However, because of the low availability of eggs, validated or not, of all species, not all egg stages were represented in all species. All participants were asked to stage all eggs, irrespective of species. The mackerel eggs in each tray were staged to IA, IB, II, III, IV, V and the horse mackerel and hake eggs were staged to IA, IB, II, III, IV, as horse mackerel and hake larvae hatch before the eggs reach stage V. Due to the fact that computers can only calculate with numeric values, stage IA was changed to 0 and stage IB to 1 in the result tables.

Each participant moved from one microscope to another in order to complete the staging and identification of all eggs. In this way, the results of the egg stage readers were not affected by differences in the quality of the microscopes. There were, however, limitations to the quality of the transmitted light source provided by two microscopes. All microscopes were fitted with eyepiece graticules.

Once each participant had staged and identified each of the eggs and the results had been entered into a result spreadsheet, a full discussion on egg staging and identification took place. From the analysis of the first set of results it became apparent which individual eggs had resulted in high or low agreement of allocated stage. Low agreement amongst participants indicated problems in allocating an egg consistently to one developmental stage. These eggs were then placed under a microscope equipped with a video camera and displayed on a large screen. Discussions then took place on the diagnostic features visible in the egg, which generally led to an agreement on the most likely developmental stage and/or species involved. In this way, the egg staging criteria (ICES, 2015b) were reviewed (see section 3.2.2).

During the course of both rounds of analysis several eggs became damaged, or were moved from one cell to another in the trays. It was therefore not possible for all participants to always stage or identify each egg. Before the second round of analysis began, another set of eggs was randomly placed in the trays. This provided a different mix of species and stages and prevented a direct comparison between the first and second round of results. However, the lessons learned during the first round of analysis and subsequent discussions would, hopefully, still be reflected in the second round results.

3.2.2 Egg staging criteria

3.2.2.1 Egg staging criteria for mackerel and horse mackerel (Western stock)

On account of discussions following the first and second round of egg staging, the participants reviewed the description of the developmental stages for mackerel, horse mackerel, hake and megrim. The primary characteristics are based on those presented in Lockwood *et al*. (1977) for mackerel (Figures 3.1 and 3.2), but also include some other (secondary) characteristics, which the participants of the previous workshops thought were crucial in determining egg stage. At this workshop it was decided that the descriptions don't need a further update. Figures 3.3 and 3.4 show the development stages for horse mackerel and figure 3.5 provides some development stages for hake eggs.

Participants should be aware that both horse mackerel and hake hatch at the end of stage 4.

Stage IA

Primary characteristics: From fertilization until cleavage produces a cell bundle in which the individual cells are not visible.

Secondary characteristics: There are no signs of a thickening of cells around the edge of the cell bundle.

NB. In preserved eggs, the edge of the cell bundle can sometimes fold over giving the appearance of a 'signet ring' seen in a stage Ib.

Stage IB

Primary characteristics: Formation of the blastodisc, visible as a 'signet ring' and subsequent thickening at one pole.

Secondary characteristics: The cell bundle has thickened around the edge giving a distinct ring appearance. Cells in the centre of the ring form a progressively thinner layer and eventually disappear.

NB. At the end of this stage, the ring can become very indistinct as it spreads towards the circumference of the egg.

Stage II

Primary characteristics: From the first sign of the primitive streak, which begins as a cleft in the cell bundle, until closure of the blastopore. Towards the end of this stage the tail tapers and is flattened against the yolk. Also at the end of this stage, the embryo should be half way around the circumference of the egg.

Secondary characteristics: Early in this stage, the primitive streak can be difficult to see, only appearing as a faint line or depression on the surface of the cell bundle. Late in this stage, the head is still narrow and the eyes are not well formed.

Stage III

Primary characteristics: The end of the tail has thickened, becoming bulbous in appearance, and may have lifted clear of the yolk-sac. Growth of the embryo is from half way to three-quarters of the way around the circumference of the egg.

Secondary characteristics: Widening of the head and development of the eyes. Pigment spots develop on the embryo.

Stage IV

Primary characteristics: Growth of the embryo from three-quarters to the full circumference of the egg.

Secondary characteristics: Eyes continue to develop and the lenses become visible. Development of the marginal fin and the tail separates from the yolk. Pigmentation on the embryo increases compared to stage 3.

Stage V

Primary characteristics: The tail of the embryo is touching the nose or beyond and circumnavigates the egg following the inner margin of the membrane.

Secondary characteristics: Pigmentation develops in the eye.

NB

The preservation of eggs can cause shrinkage and distortion of the embryo. Therefore, care should be taken when assessing the length of the embryo, as they do not always remain around the full circumference of the egg. The embryo may also become distorted giving a false impression of development stage.

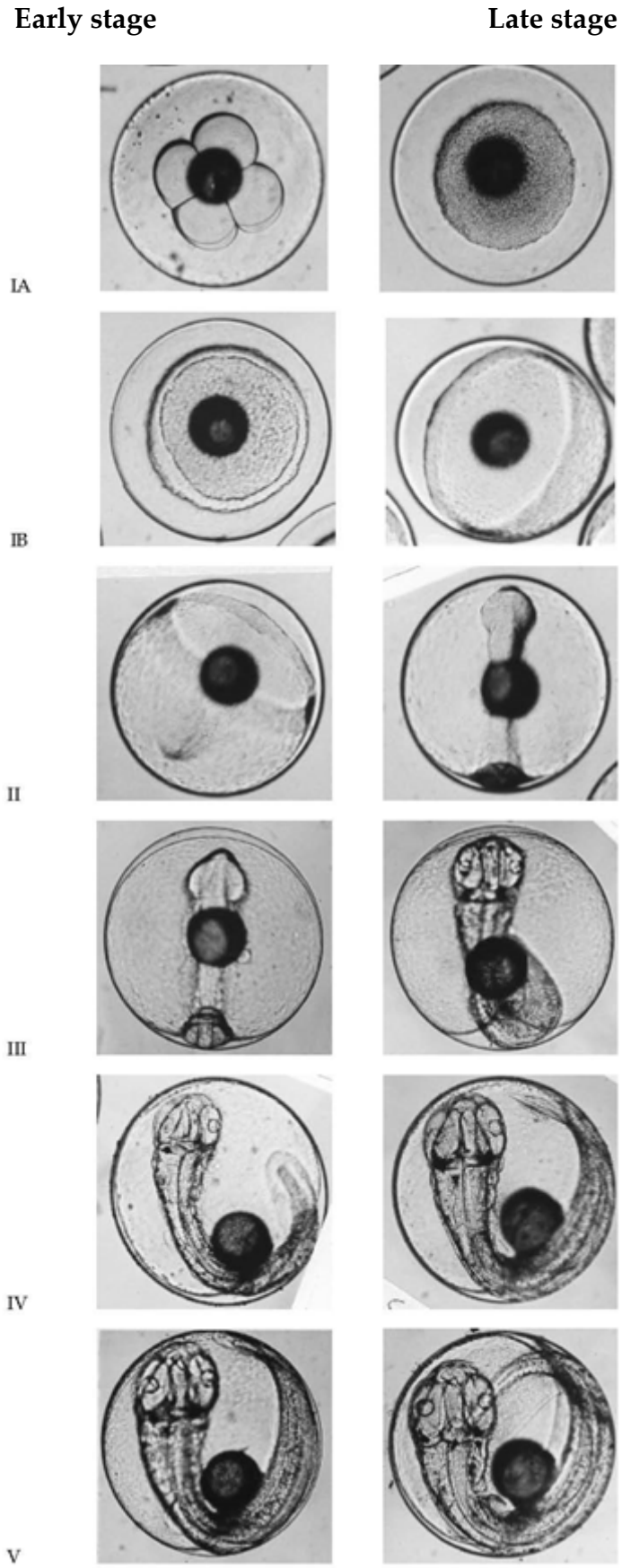
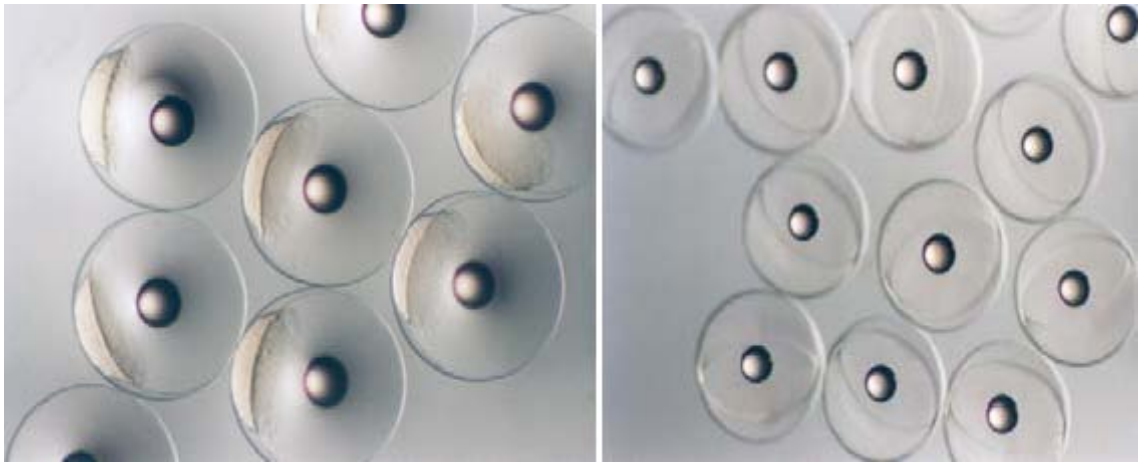
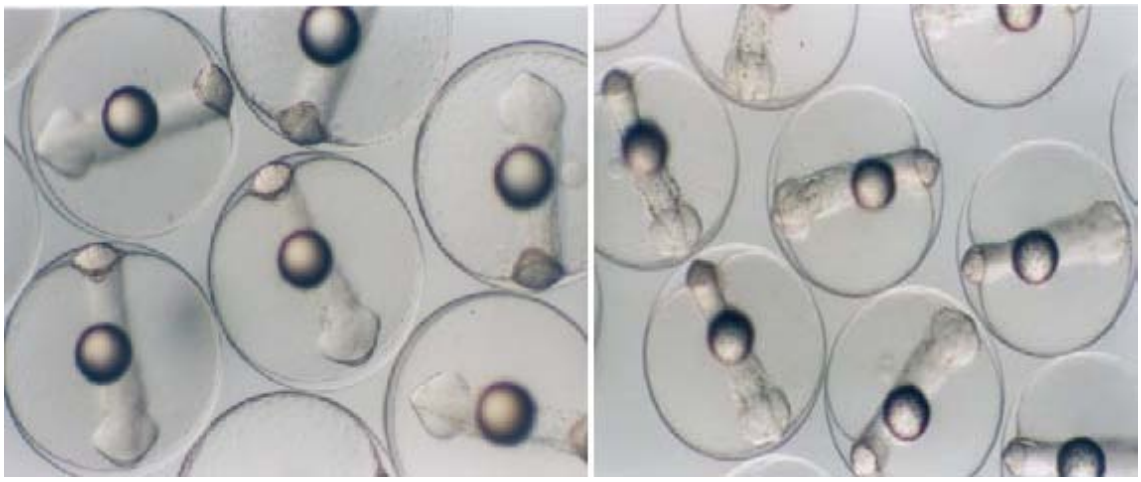


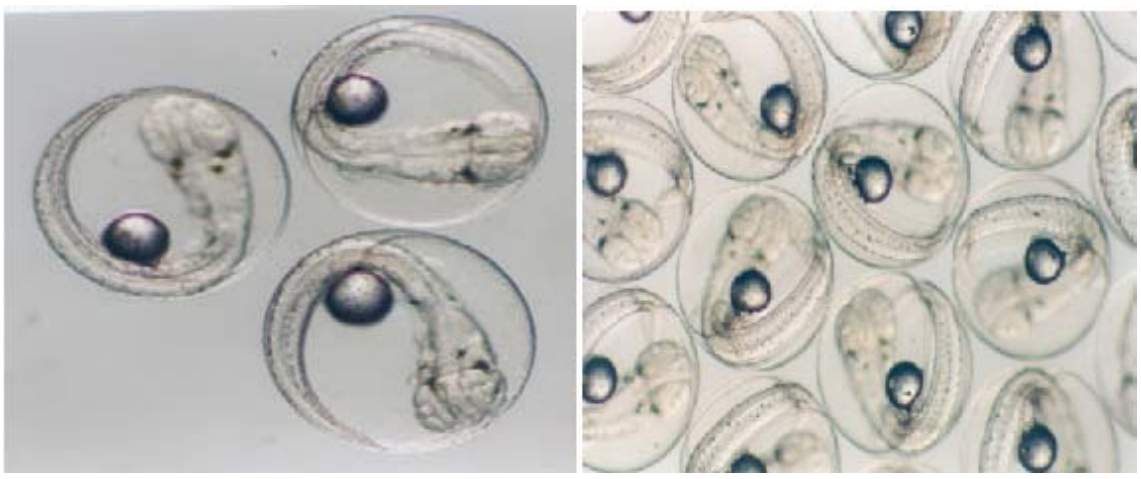
Figure 3.1. Mackerel eggs at the beginning and end of the six development stages.



Stage 1A Stage 1B



Stage II Stage III



Stage IV Stage V

Figure 3.2. Development stages of mackerel from fertilization experiments.

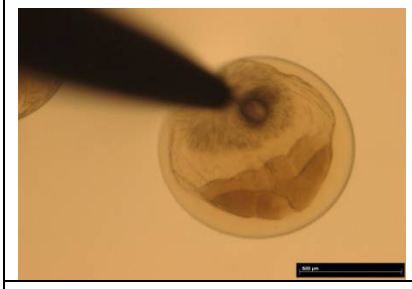
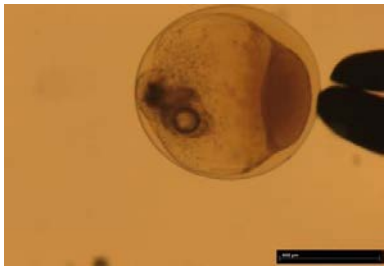
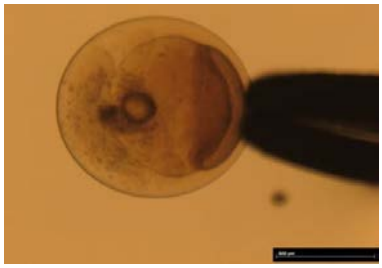
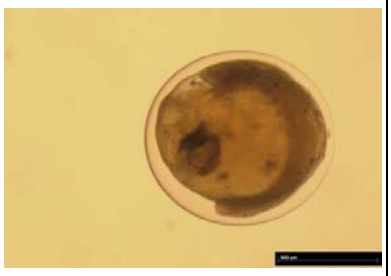
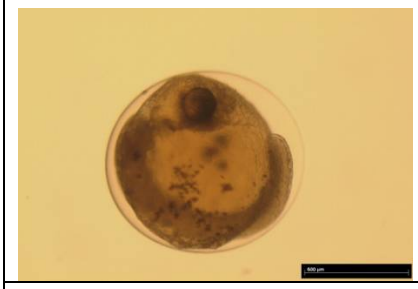
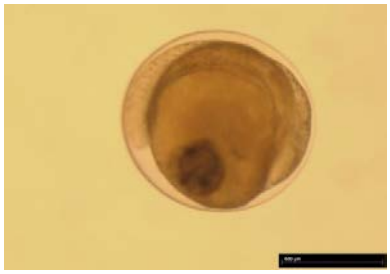
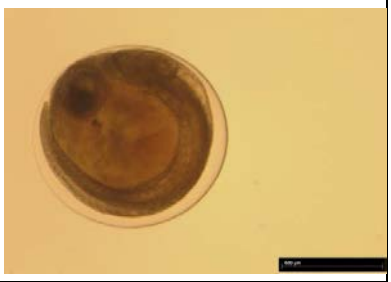
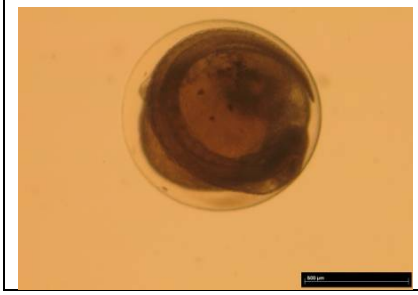
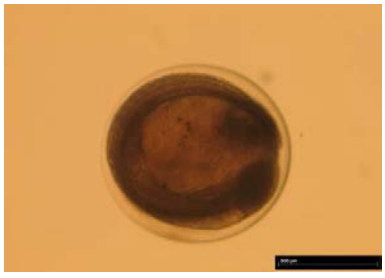
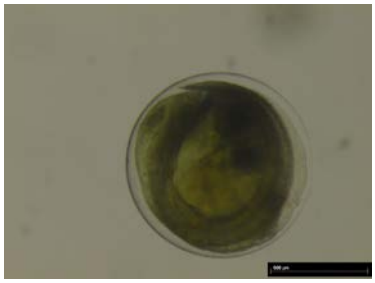
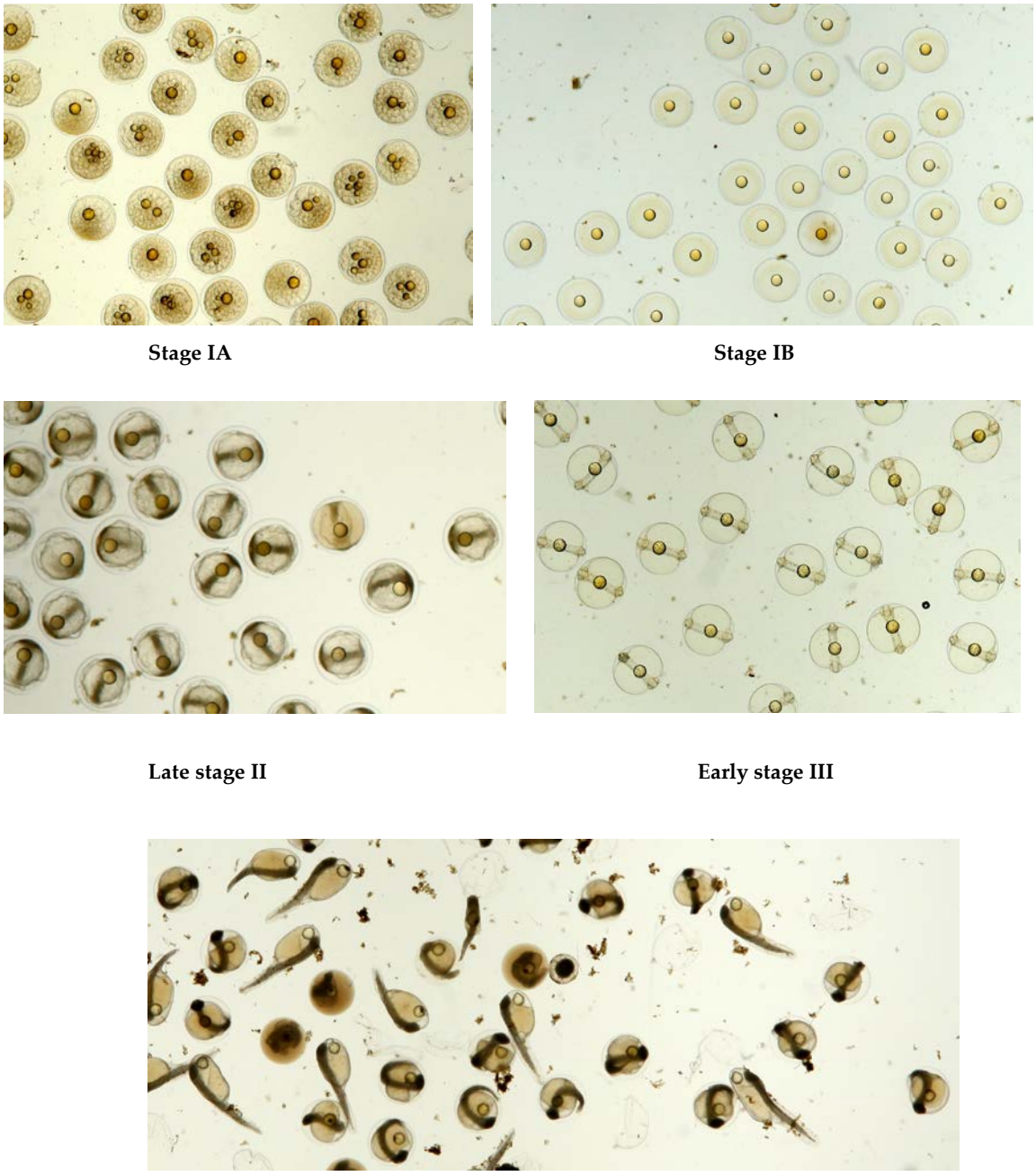
Stage IA or I	Stage IA or II	Stage IB or III
		
Stage II or IV	Stage II or V	Stage III or VI
		
Stage III or VII	Stage III or VIII	Stage IV or IX
		
Stage IV or X	Stage IV or X	Stage IV or XI
		

Figure 3.3. Development stages of horse mackerel from fertilization experiments. First stage number is the stage development used for the Western stock, second number is the stage development used for the Southern stock.

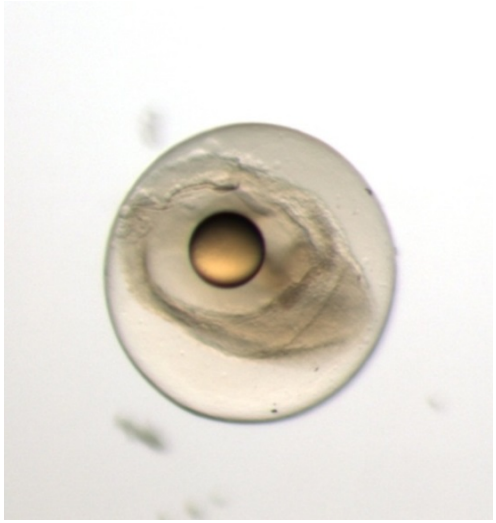


Stage IA **Stage IB**

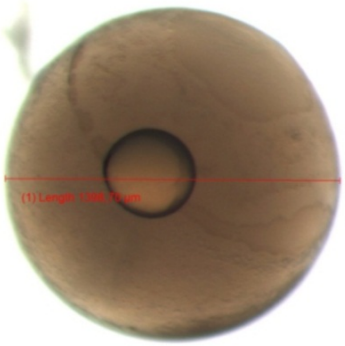
Late stage II **Early stage III**

Late stage IV and hatching

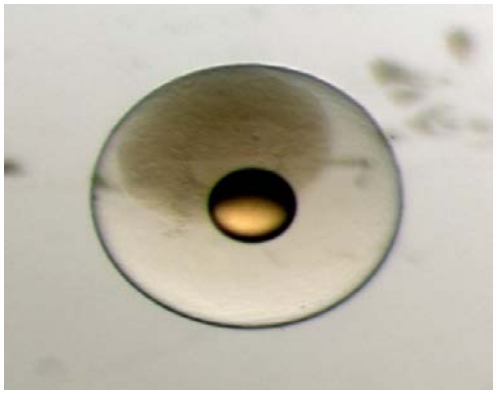
Figure 3.4 Development stages of horse mackerel from fertilization experiments.



Stage 1A

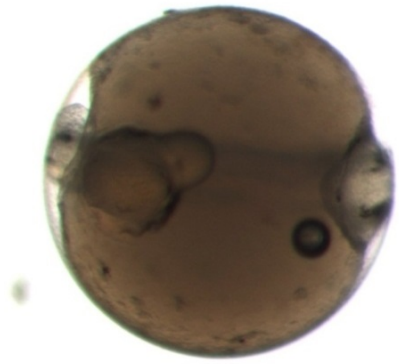


Stage 1A

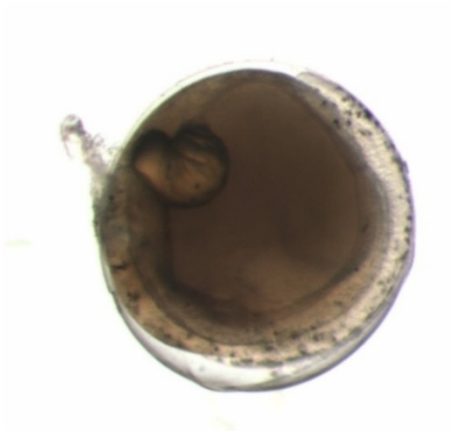


Stage 1B

Stage II



Stage III



Stage III

Figure 3.5. Development stages of hake eggs from fertilization experiments.

3.3 Egg identification (ToR c, d and e)

3.3.1 Egg identification trials

The same trays of fish eggs (described in section 3.2 above) were also used for the egg identification exercise. As each participant moved from microscope to microscope they were asked to provide a species identification for each egg, in addition to a development stage. The descriptions of the different species from the 2015 workshop report (ICES, 2015b) was available to participants prior to the first staging round.

The results of the first round of egg identifications were collated and input into spreadsheets at the same time as the results for egg staging. The results were presented and eggs with low agreement in species identification were displayed on a large screen (as described in section 3.2 above). A discussion then took place until a consensus was reached on the most likely species identification for each of these eggs. As a result of these discussions and before the second round of analysis was begun, a review of the egg identification criteria produced by previous WKFATHOM participants was carried out. It was decided that the descriptions of the target and of most other very similar species didn't need to be udated in the survey manual. Howerver, there was a longer discussion on the possible confusion of eggs of mackerel with those of ling, particularly in the northern part of the survey area. An update on the problems with the discrimination of ling from mackerel eggs was added to the survey manual.

3.3.2 Egg identification criteria

Egg and oil globule size are the primary criteria used in identification of eggs. Mackerel eggs range in size from 0.97 mm to 1.38 mm with the oil globule ranging from 0.22 to 0.38 mm. Horse mackerel eggs range from 0.81 to 1.04 mm with an oil globule ranging from 0.19 to 0.28 mm.

Table 3.1 summarizes published descriptions of mackerel, horse mackerel and other species of eggs that contain similar morphological features. It provides validated observed egg and oil globule diameters for each species as well as the diagnostic features and criteria used by the participants to help with egg identification. It should be noted that the diameter of the egg and oil globule within a species can and may vary through the spawning season and also from area to area. Variation in egg size for the same species can also be observed within the same sample.

Eggs may also show regional variations in pigmentation and this should not therefore be used as a primary characteristic for identification. Due to this variation, egg identification should be carried out only by experienced staff that have participated in the WKFATHOM egg identification and staging workshops carried out in the year prior to the survey year.

Table 3.1. Comparison of the Characteristics of Mackerel, Horse Mackerel, Blue Jack Mackerel, Megrim, Hake and Snipefish Eggs (Details of fixative and concentration unknown). NB The information in Table 9.2.1 above is based on observations of live or recently preserved eggs. It must be noted that preservation in formaldehyde gradually destroys pigmentation and therefore observation of chromatophores may well be difficult in specimens, which have been preserved for any length of time.

Species	Diameter (mm)		Reference	Area	Diagnostic Features
	Egg	Oil Globule			
Mackerel (*Scomber scombrus*) (See Lockwood *et al.*, 1977)	1.0-1.38	0.28-0.35	Russell, 1976	North Sea, English Channel	• Unsegmented/ Homogenous yolk • Perivitelline space approx. 0.05mm • Oil globule often orientated to the top of the egg
	1.09-1.36	0.26-0.37	Fahay, 1983	N.W. Atlantic	
	0.97-1.38	0.25-0.35	Ehrenbaum, 1905-09	Irish Sea, North Sea	
	1.24	?	Mendiola *et al.*, 2006	Biscay	
	0.97-1.38	0.22-0.38	Development of Fishes of the Mid-Atlantic Bight, 1978	Mid-Atlantic Bight	
	1.0-1.38			North Atlantic	
	0.97-1.38	?	Johnstone, Scott and Chadwick, 1934	Isle of Man	
	1.21-1.33	~0.32	Holt, 1893	West of Ireland	
	1.16	0.27	IPIMAR, fertilization experiment 2008		
Horse Mackerel (*Trachurus trachurus*)	0.81-1.04	0.19-0.28	Russell, 1976	North Sea, English Channel	• Granular / segmented yolk, although this may not be as obvious at the southern end of the species range. • The oil globule migrates towards the head of the embryo after stage 2.
	1.03-1.09	0.26-0.27	Holt, 1898	North Sea	
	0.81-0.93	0.22-0.23		Plymouth	

Species	Diameter (mm)		Reference	Area	Diagnostic Features
	Egg	Oil Globule			
(See Pipe and Walker, 1987)	0.84-1.04	0.19-0.24	Ehrenbaum, 1905-09	North Sea, English Channel	• In stages 3 and 4 the embryos show stronger pigmentation compared to mackerel. However, the pigmentation is not as strong as in hake. • Oil globule easily broken into several smaller pieces. This seems to be more common in eggs found in the southern area, particularly in eggs from the Portuguese coast.
	Max. 0.84	0.24-0.26	Holt, 1893	English Channel	
Blue Jack Mackerel (*Trachurus picturatus*)	0.98-1.10	0.19-0.31	IPIMAR, fertilization experiment 2010 (Gonçalves *et al.*, 2012)	W Portugal	• Segmented yolk
Megrim (*Lepidorhombus whiffiagonis*)	1.02-1.22	0.25-0.30	Russell, 1976	North Sea, Irish Sea	• Striated appearance of egg membrane*. (See below and Figure 3.7) • Oil globule is closer to egg membrane than in mackerel.
	1.07-1.22	0.25-0.30	Ehrenbaum, 1905-09	North Sea	
	1.07-1.13	0.30	Holt, 1893	West of Ireland	

Species	Diameter (mm)		Reference	Area	Diagnostic Features
	Egg	Oil Globule			
	1.08-1.30	0.29-0.34	CEFAS unpublished data	Celtic Sea	• Embryo thinner than a mackerel embryo. • Yolk unsegmented and the egg has a small perivitelline space. • Pigmentation on yolk from stage II onwards. • Pigment on oil globule as embryo develops ***Striations can be observed on the membranes of preserved eggs of other species. This can lead to misidentification of eggs which have been preserved for some time.**
Hake (*Merluccius merluccius*) (See Coombs, 1982)	0.94-1.03	0.25-0.28	Russell, 1976	North Sea, English Channel, Mediterranean	• Positive surface adhesion test (SAT) is used to identify hake eggs (Porebski, 1975) and (Coombs, 1994). • From stage III onwards embryos display strong pigmentation along the embryo. Towards the end of its development, the embryo begins to show the characteristic post-anal pigmentation of three bars.
	0.94-1.03	~0.27	Ehrenbaum, 1905-09	North Sea, English Channel, Mediterranean	
	0.94-1.03	~0.27	D'Ancona *et al.*, 1956	?	
	1.10-1.16	0.27-0.35	Shaw, 2003	Celtic Sea	

Species	Diameter (mm)		Reference	Area	Diagnostic Features
	Egg	Oil Globule			
Longspine Snipefish *(Macrorhamphosus scolopax)*	1.00	0.2	Development of Fishes of the Mid-Atlantic Bight, 1978. U.S. Fish and Wildlife service. FWS/OBS-78/12.	Europe	• Membrane is light amber with grainy reflections • Yolk with rose or violet halo depending on viewing light. • Oil globule is amber/rose in colour
Lings (Molva spp.)	0.97 – 1.13	0.28 – 0.31	Russell, 1976	North Sea	• Unsegmented yolk • Pigmented oil globule • Pigmentation in later stage embryo is concentrated into 2 distinct lines that run all the way along the back. • Most likely to occur in temperatures < 8.5 °C

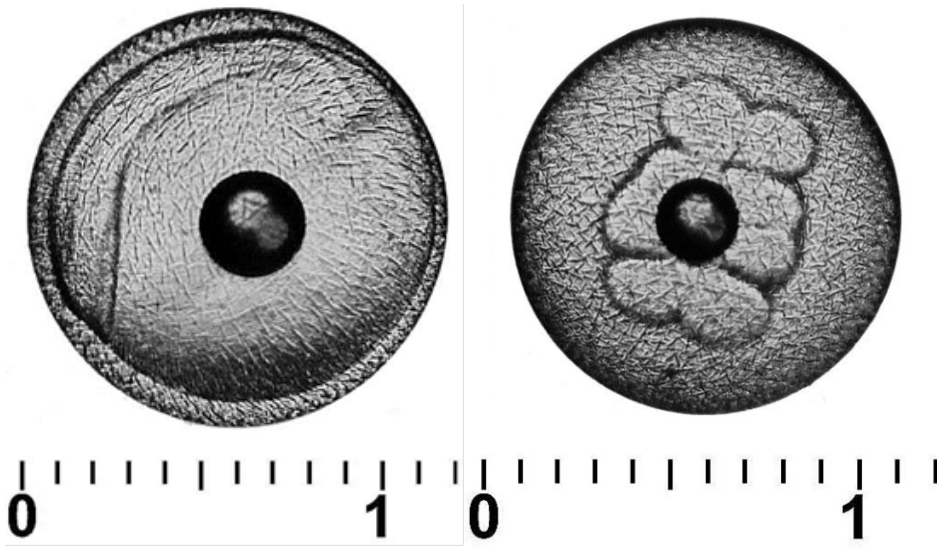


Figure 3.6. Eggs of megrim, showing the striations on the membrane.

3.3.3 Misclassification of mackerel and horse mackerel eggs in ICES Division IXa

In the southern part of the area of the triennial mackerel and horse mackerel egg survey different species of mackerel (*Scomber scombrus* and *S. colias*) and horse mackerel (*Trachurus trachurus*, *T. mediterraneus* and *T. picturatus*) occur. The species of each genus show overlapping distributions and spawning periods and their eggs are similar in morphology. In order to help in the identification of these species, descriptions of morphometric characteristics of these eggs and the most relevant aspects for their identification are given below:

Trachurus mediterraneus

- Egg diameter: 1.00–1.04 mm
- Oil globule: 0.24 mm
- Description: Pelagic eggs, spherical, transparent. No perivitelline space. Oil globule colourless. Fine striated membrane (Padoa, 1956).
- Eggs are similar to *Trachurus trachurus*, but a bit bigger.
- Distribution of adults appears in the reports of ICES-WGACEGG.

Trachurus picturatus

Description and measurements based on eggs from a single artificial fertilization experiment carried out in 2010 by IPMA (Figure 3.7).

- Pelagic, spherical and transparent eggs with a small perivitelline space. The yolk-sac is segmented. A single yellow oil globule is located towards the posterior portion of the yolk. In the early embryo, two rows of spots appear along the dorsal body contour.
- Eggs are very similar to the eggs of *Trachurus trachurus*. The *T. picturatus* eggs from the 2010 fertilization experiment were slightly larger than the eggs of *T. trachurus* described in the literature and exhibited a more intense pigmentation.
- Egg diameter: 0.98 – 1.10 mm
- Oil globule: 0.19 – 0.31 mm

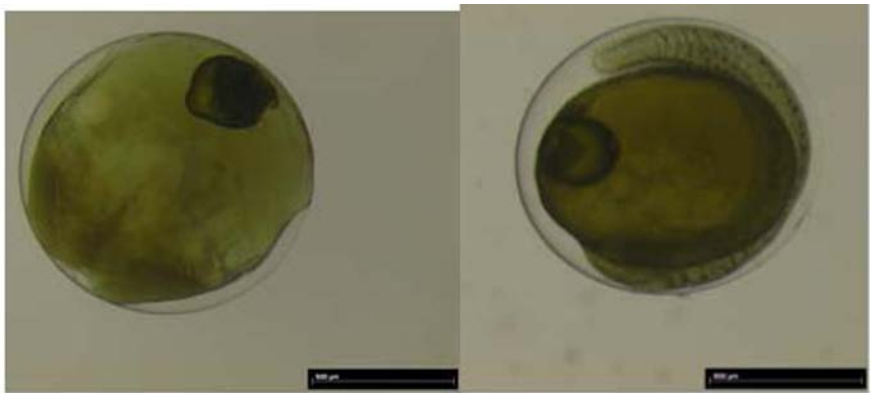


Figure 3.7. Eggs of *Trachurus picturatus* from a fertilization experiment (IPMA, 2010)

Scomber colias

- The eggs are spherical, on average ranging in diameter from 1.06–1.14 mm. Similar description was offered by Fahay (1983), with little differences in diameter range, which ranged from 1.06–1.36 mm.
- Oil globule 0.26–0.37 mm in diameter. In the Pacific oil globules diameters varies between 0.25 and 0.32 mm (Fritzsche, 1978).
- Yolk is smooth, transparent and unsegmented and under magnification (x36) can be seen to be filled with a large number of tiny vacuoles. The only difference with *S. scombrus* is that the yolk is pigmented with several melanophores, while in *S. scombrus* eggs the yolk is pigmented just before hatching, when a spot per side appears just posterior to the head.
- The perivitelline space is narrow.
- In advanced stage of development both the dorsum of the embryo and the oil globule are pigmented, the latter on the hemisphere facing the head (Kramer, 1960).
- Distribution of adults appears in the reports of ICES-WGACEGG.

Macroramphosus scolopax

- Egg diameter: 1.0 mm
- Oil globule: 0.20 mm
- Description: Pelagic eggs, spherical, transparent, single oil globule. Yolk pigmentation is described as light amber; pigmentation of oil globule is amber-rose (Spartà, 1936). Eggs are similar to those of *Trachurus trachurus* but without yolk segmentation.
- For fish distributions see for example Marques *et al.* (2005).

Boops boops

- Egg diameter: 0.93 mm (based on eggs from artificial fertilization, IPMA, 2008, see Figure 3.8).

- Oil globule: 0.18 mm (based on eggs from artificial fertilization, IPMA, 2008).
- Description: Pelagic eggs, spherical. Single oil globule with melanophores (Gaetani, 1937).
- Fish distribution is mapped in the reports of ICES-WGACEGG.

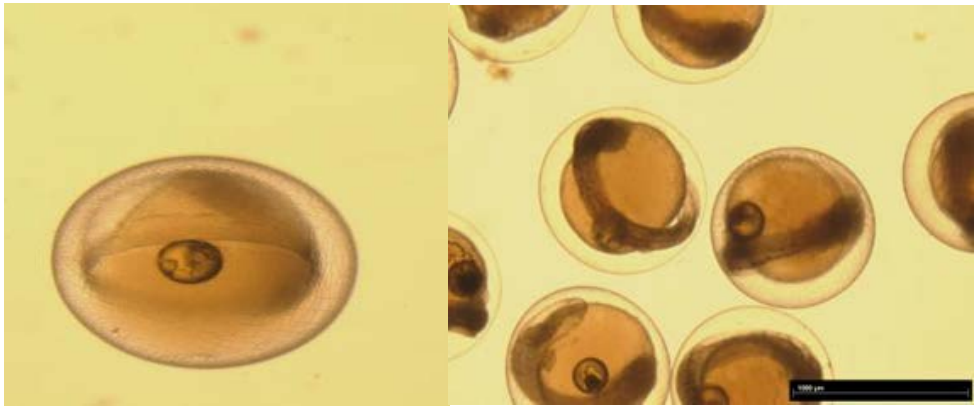


Figure 3.8. Eggs of *Boops boops* from fertilization experiments (IPMA).

3.4 Inter-calibration work on fecundity, atresia and POF staging (ToR e)

The work was organized to calibrate the measurement and analysis of the parameters that are estimated in both ADEPM and DEPM respectively.

Previous to the workshop participants were encouraged to download from the shared FTP all material that was going to be used during the workshop, i.e. pictures of histological slides, image processing program with the ObjectJ plugin along with *.ojj projects, ND2.view software viewer, the manuals and the templates.

All the pictures in the different exercises came from samples collected during the survey 2016, except in the fixative exercise. The slides in the screening and POF staging exercises were scanned by IMR while the same pictures taken during the survey year were used in the atresia and fecundity exercises. 17, 15, 6 and 5 samples were available for POF staging, screening, atresia and fecundity exercises respectively.

Particularly, the fixative exercise consisted of discussing in plenary the results obtained in a comparative study carried out on fixation of materials with the formaline-free fixative "Finefix" and formaline for frozen and fresh gonad samples.

Finally, participants were suggested to bring the micropipettes to the workshop for a trial with fresh gonads in the laboratory.

3.4.1 Standardization of ovary screening and analysis

Before starting the exercise, descriptions of Hydration_stage, Egg_stage, Early_alpha_atresia, Massive_atresia, Spent and POF was discussed, in order to harmonize both the classification of the ovary and the interpretation of the template among participants.

In relation to the screening exercise, it was discussed in plenary whether the spoon subampling was satisfactory, because there is a concern this sampling may not be adequate when looking for certain stages of oocyte development, i.e. related to hydration, and POFs.

New to this workshop was the usage of NDP.view2 for analysing the screening slides. After a quick explanation from IMR all participants agreed that it is very easy to work with the program and a great tool also for the workshop.

Results are presented in section 4.4.1.

3.4.2 Standardization of POF staging criteria and analysis

The POF staging criteria for identifying daily cohorts of spawners was reviewed before the exercise. It is an issue than remains difficult to harmonize among readers. It was agreed that indicators were needed to help decision-making in those POFs in transition between stages. The criteria on the number as well as the size of POFs were discussed to be a valid indicator, so it was decided to update the POFs staging criteria by modifying the description and pictures and adding key characteristics.

NDP.view2 viewer was used to carry out the POF staging exercise.

Results are presented in section 4.4.2.

3.4.3 Standardization of alpha atresia criteria and intensity image analysis

The early alpha atresia description was first addressed in order to harmonize the criteria among participants. It was agreed that the description of atresia in the manual was not detailed enough, i.e. it was not clear whether both chorion layers should have a break more than twice the width to be classified as late alpha and needed further revision. It was decided that if either chorion layer has any break more than twice its width is classified as late atresia and it is no scored then.

ImageJ image analysis program with the ObjectJ plugin was used in this exercise after a presentation of IMR on the use of this tools.

Results are presented in section 4.4.3.

3.4.4 Standardization of potential fecundity image analysis

Each reader scored the whole mount images using ImageJ with the ObjectJ plugin following the instruction received before the exercise on the use of this tool.

Results are presented in section 4.4.4.

3.4.5 Standardization of micropipette sampling

During the workshop micropipette sampling trials were carried out with fresh plaice females, with ovaries at the late developing stage. Fresh mackerel gonads were not available at the time of the workshop. Some participants brought their institute pipette and plunger to test for their adequacy. Two Wiretrol micropipette sizes (50 and 100 microliters) were tried for the ovary sampling.

4 Results

4.1 Results of egg sorting exercise

Two plankton sample rounds were prepared with a known number of fish eggs (a mix of mackerel and hake eggs typical for survey samples) present in each. Table 4.1.1 shows the numbers of eggs removed by each use of the spray technique for the first, second, and third round. Most of the eggs, which were present in the samples, were removed during the first spray, improving the eggs extraction from the first to the third round from 51% to 68% respectively (Figure 4.1.1). During the second round, the extraction of eggs was considerably higher already after the first spray and increased to almost 75 % after 3 spray extractions. In general, there were no wider fluctuations (CV=30%) in determining the number of eggs among participants despite of the inexperience of some of them (Figure 4.1.2). However, the spraying results became more consistent among participants during the second spraying exercise (Figure 4.1.2). At the end of the exercise, almost all participants removed more than 90% of eggs occurred in the samples.

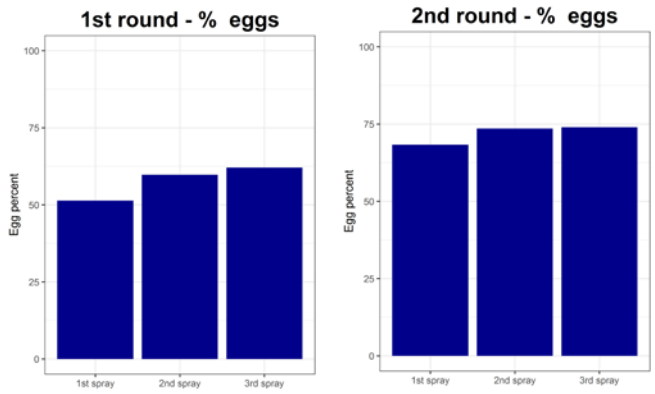


Figure 4.1.1: Cumulative percentage of eggs removed from the plankton samples for 1st round (left) and 2nd round (right).

Table 4.1.1: Results of the exercise of the spray technique to remove fish eggs from plankton samples for each team and the average values for

Fish Eggs	Round 1										
Participants	**Team 1**	**Team 2**	**Team 3**	**Team 4**	**Team 5**	**Team 6**	**Team 7**	**Team 8**	**Team 9**	**Team 10**	**Average**
# Eggs					130						
Spray #1	57	52	62	88	56	52	66	59	83	93	
Spray #2	25	5	23	20	4	6	4	13	1	8	
Spray #3	1	3	1	1	3	8	1	1	11	0	
Total Spray	83	60	86	109	63	66	71	73	95	101	
%removed spray	64%	46%	66%	84%	48%	51%	55%	56%	73%	78%	**62%**
Rest	40	67	42	22	62	54	52	52	33	30	45
TOTAL removed	123	127	128	131	125	120	123	125	128	131	**126**
%removed	95%	98%	98%	101%	96%	92%	95%	96%	98%	101%	**97%**

Fish Eggs	Round 2										
Participants	**Team 1**	**Team 3**	**Team 5**	**Team 9**	**Team 10**	**Team 2**	**Team 4**	**Team 6**	**Team 7**	**Team 8**	**Average**
# Eggs			**150**					**131**			
Spray #1	87	98	95	90	93	102	99	102	87	101	
Spray #2	3	11	12	8	5	3	6	4	16	5	
Spray #3	0	3	0	3	0	0	0	0	0	0	
Total Spray	90	112	107	98	98	105	105	106	106	101	
%removed spray	60%	75%	71%	67%	65%	80%	80%	81%	79%	81%	**74%**
Rest	44	35	38	42	40	18	10	6	22	18	**27**
TOTAL removed	134	147	145	143	138	123	115	112	125	124	**131**
%removed	89%	98%	97%	95%	92%	94%	88%	85%	95%	95%	**93%**

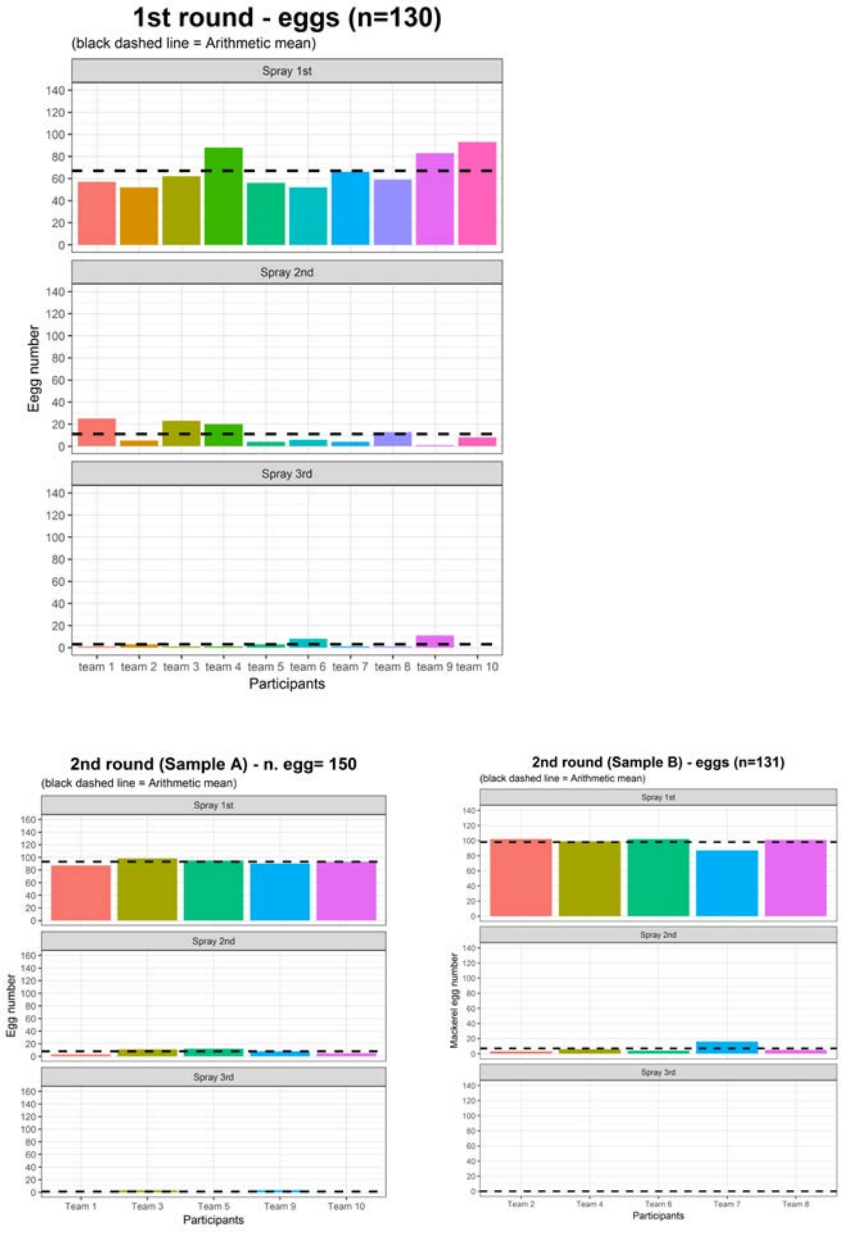


Figure 4.2: Number of eggs removed from the plankton samples using spray technique by participants for 1st round (upper) and 2nd round (lower). Black dashed line represents the arithmetic mean of the values.

Special attention was paid to the hake eggs due to the hydrofuge nature of their chorion. These eggs tend to cling to the air-water-interface and are, thus less susceptible to the spray method while staying afloat with the other plankton through the introduction of air bubbles. Figure 4.1.3 shows the number of floating eggs identified during the exercise by spray and participant. Results obtained in the 1st round are presented by each use of the spray technique (left) but in the 2nd round results are presented once the three sprays had been ended (right).

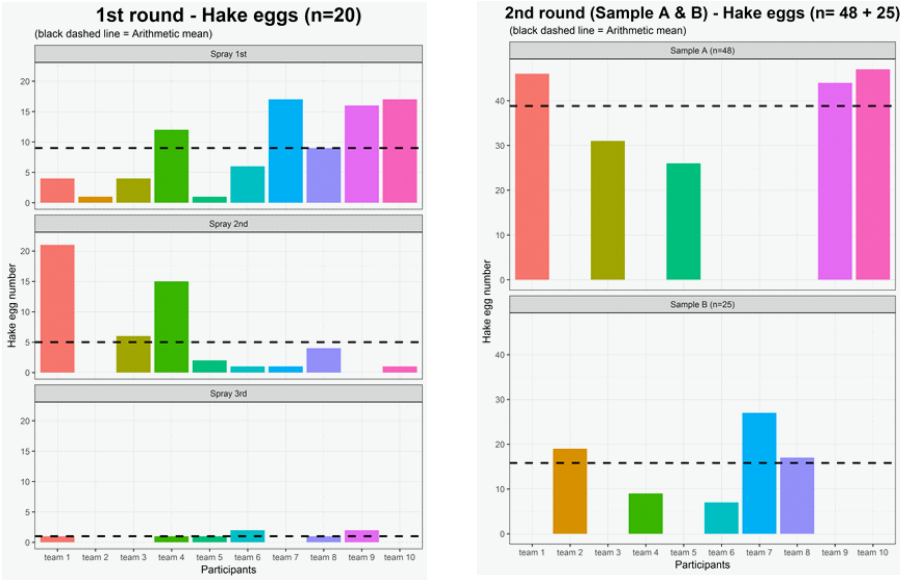


Figure 4.3: Number of floating eggs identified from the plankton samples for each use of spray technique by participants in 1st and 2nd rounds. Black dashed line represents the arithmetic mean of the values.

There is a high variability (>70%) in the extraction of hake eggs between participants. Here, the experience of the participants seems to play a significant role in the identification: Not all floating eggs are hake, but only those that float as described in Porebsky (1975). Additionally, the final identification of these eggs has to be confirmed under the binocular, bearing in mind other characteristics such as the egg diameter and pigmentation of the embryo, if present. However, the exercise showed that the spray technique is in deed less efficient for hake eggs than for mackerel and horse mackerel eggs (Figure 4.4).

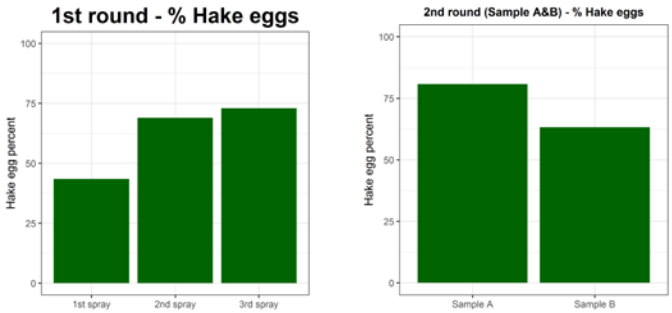


Figure 4.4. Cumulative percentage by spray of identified hake eggs from the plankton samples for 1st round (left) and total percentage by sample of identified hake eggs from the plankton samples for 2nd round (right).

The results obtained corroborate the effectiveness of the spray technique in the separation of fish eggs from plankton. The method speeds up the fish egg separation process (75% after the 3rd spray) and is specially recommended for those plankton samples with high egg densities.

4.2 Result of egg staging exercises

The results of the egg staging exercises are given in Tables 4.2.1 to 4.2.12.

Tables 4.2.1 to 4.2.3 presents the results for each participant for the first round of analysis for eggs of all species (Table 4.2.1), for mackerel eggs (Table 4.2.2) and for horse mackerel eggs (Table 4.2.3). About half of the participants at the workshop were inexperienced; hence results of only the expert readers are presented separately (Table 4.2.4 – 4.2.6). Tables 4.2.7 to 4.2.12 presents the results for the second round of analysis in exactly the same way.

The original assessment of each egg, by each participant, for stage (and species), was input into a primary result table (not presented here). Once the results were available from every participant a modal stage could be calculated for each unvalidated egg (i.e. those not from fertilization experiments). This modal assessment of egg stage was presumed to be 'correct' although it does not necessarily mean that this was the true stage.

Tables 4.2.1 to 4.2.12 summarize the results into six sub-tables labelled A-F, where the performance of each participant is judged against the modal egg stage.

Sub-tables A show the number of eggs at each modal stage that were assessed by each participant. The numbers at each modal stage will therefore be the same for all participants that read all the eggs.

Sub-tables B show the numbers of eggs at each stage as assessed by each participant.

Sub-tables C show the over / underestimation of stage 1 (1a + 1b) by each participant.

Sub-tables D show how well each participant's assessment of egg stage agrees with the numbers of eggs at each model stage.

Sub-tables E show the percentage agreement of each participant's assessment of eggs in stage 1a+1b against the modal stage 1a+1b.

Sub-tables F show the bias of each participant's egg staging against the modal stage i.e. how much their assessment of each egg stage varies from the modal stage.

By studying the results presented in Tables 4.2.1 to 4.2.12, some encouraging improvements in the consistency of egg staging between participants can be observed from the first to the second round of analysis.

The overall agreement in egg stage for all species of eggs, in all stages of development was 65 % in the first round (Table 4.2.1). This increased to 78 % agreement in the second round of analysis (Table 4.2.7). The agreement between the expert readers was higher compared to overall and increased from 72 % to 83 % (Table 4.2.2 and 4.2.8). The overall agreement for all egg stages, for mackerel, increased from 64 % (Table 4.2.3) to 79 % (Table 4.2.9), for horse mackerel however, the score decreased from 87 % (Table 4.2.5) to 82 % (Table 4.2.11). For the experts agreement for all egg stages, for mackerel, increased from 70 % (Table 4.2.4) to 82 % (Table 4.2.10), and for horse mackerel it decreased from 99 % to 87 % (Table 4.2.6 and 4.2.12). The horse mackerel results need to be treated with care, because only very few eggs of the various stages of horse mackerel were available for this staging exercise. In particular, the older stages were mostly lacking in the two setups.

The overall agreement for stage 1 (1a+1b) eggs, the most critical stages for the calculation of the annual egg production in both target species, showed improvements with an overall greater level of agreement, from 90 % in the first round to 99 % in the second round. (Tables 4.2.1 and 4.2.7). Agreement between the experts increased from 96 to 99 % (Tables 4.2.2 and 4.2.8). The overall agreement of stage 1 eggs, for mackerel, increased from 89 % (Table 4.2.3) to 100 % (Table 4.2.9), and for horse mackerel from 97% (Table 4.2.5) to 98% (Tables 4.2.11). For experts agreement of stage 1 eggs, for mackerel,

increased from 95% (Table 4.2.4) to 100% (Table 4.2.10), and for horse mackerel remained at the same level 99% (Tables 4.2.6 and 4.2.12).

The percentage agreement in allocating eggs to stage 1 (1a+1b) as a percentage over- or underestimation, are given in sub-tables C. Although the overall bias was reasonable, particularly in the first round of analysis, some individuals showed very high levels of bias. In the first round of analysis there was no overall bias with a mean over- or underestimation of 0% for eggs of all species but individual bias ranged from an underestimate of -28% to an overestimate of 12% (Table 4.2.1). In the second round there was a slight overall overestimation of 2 %, but the range of individual bias reduced to between -1% to 6% (Table 4.2.7). For the experts the overall bias was an overestimate of 2% for eggs of all species in both rounds but individual bias ranged from an underestimate of -1% to an overestimate of 8% (Table 4.2.2) in the first round. In the second round the range of individual bias was reduced to between 0% and 5% (Table 4.2.8).

The mean over- or underestimation for stage 1 mackerel eggs (Tables 4.2.3 and 4.2.9) was -6 % in the first round and 2% in the second round of analysis. However, the bias of individual participants was much greater, ranging from -46% to 13% in the first round, but improving to between 0% to 7% in the second round of analysis. For experts the overall bias for mackerel stage 1 was -2% in the first round and 1% in the second (Tables 4.2.4 and 4.2.10). Individual bias ranged from -7% to 1% and narrowed to 0% to 4% in the second round. The overall bias for stage 1 horse mackerel eggs (Tables 4.2.5 and 4.2.11) was 16% in the first round and fell to 1% in the second round of analysis. However, the bias of individual participants was again much greater, ranging from -62% to 18% in the first round, but improving to between -8% and 25% in the second round of analysis. For experts the overall bias for horse mackerel stage 1 was 1% and 2% in the first and second round, respectively (Tables 4.2.6 and 4.2.12). Individual bias for horse mackerel in the first round ranged from -7% to 0% and deteriorated from --8% to 25%.

Table 4.2.1. All eggs first staging. (A) The numbers of eggs at each modal stage read by each participant. (B) The numbers of eggs allocated to each stage by each participant. (C) The over / underestimation of stage 1 (1a+1b) by each participant. (D) The percentage agreement by modal egg stage by each participant. (E) The percentage agreement by modal stage 1a and 1b combined, by each participant. (F) The bias is indicated by the percentage over or underestimation of each egg stage, as estimated by each participant, in relation to the modal stage. For each table the combined result is also given.

ALL EGGS first stageing Egg Stageing Workshop Bremerhaven, October 2018

A — NUMBER OF EGG STAGE READINGS BY MODAL EGG STAGE

	MODAL stage	Reader 1	Reader 2	Reader 3	Reader 4	Reader 5	Reader 6	Reader 7	Reader 8	Reader 9	Reader 10	Reader 11	Reader 12	Reader 13	Reader 14	Reader 15	Reader 16	Reader 17	Reader 18	Reader 19	Reader 20	Reader 21	TOTAL
Stage 1a ==>	0	108	108	109	109	108	108	109	109	109	110	104	109	110	110	110	108	108	110	108	110	105	2279
Stage 1b ==>	1	49	48	49	49	49	49	49	49	49	49	46	49	49	49	49	48	49	49	49	49	49	1024
Stage 2 ==>	2	36	36	36	36	36	36	36	36	37	36	36	37	36	36	36	36	37	36	36	36	36	759
Stage 3 ==>	3	43	43	43	43	43	43	43	43	43	42	41	42	43	43	43	43	43	43	40	43	42	895
Stage 4 ==>	4	60	60	60	60	59	60	60	60	60	60	56	60	60	60	60	60	60	59	58	60	58	1250
Stage 5 ==>	5																						-
Total	0-5	296	295	297	297	295	296	297	297	298	297	283	297	298	298	298	295	297	297	291	298	290	6207

B — EGG STAGE COMPOSITION

	Stage	Reader 1	Reader 2	Reader 3	Reader 4	Reader 5	Reader 6	Reader 7	Reader 8	Reader 9	Reader 10	Reader 11	Reader 12	Reader 13	Reader 14	Reader 15	Reader 16	Reader 17	Reader 18	Reader 19	Reader 20	Reader 21	TOTAL
Stage 1a ==>	0	158	55	152	129	117	140	79	109	95	157	158	100	107	83	105	122	88	90	59	125	100	2328
Stage 1b ==>	1	0	76	10	43	41	21	73	50	79	1	1	42	8	79	43	46	63	77	98	40	72	963
Stage 2 ==>	2	36	61	32	28	39	29	38	33	32	31	28	67	78	31	39	19	47	27	35	25	23	778
Stage 3 ==>	3	55	27	56	41	48	38	62	32	36	59	38	47	49	54	34	22	51	60	46	55	70	980
Stage 4 ==>	4	45	51	19	49	38	58	40	59	34	44	52	27	49	49	56	68	34	26	48	44	22	912
Stage 5 ==>	5	2	25	28	7	12	10	5	14	22	5	6	14	7	2	21	18	14	17	5	9	3	246
Total	0-5	296	295	297	297	295	296	297	297	298	297	283	297	298	298	298	295	297	297	291	298	290	6207

C — OVER- / UNDERESTIMATION OF STAGE 1 (=1A+1B)

MODAL stage	Reader 1	Reader 2	Reader 3	Reader 4	Reader 5	Reader 6	Reader 7	Reader 8	Reader 9	Reader 10	Reader 11	Reader 12	Reader 13	Reader 14	Reader 15	Reader 16	Reader 17	Reader 18	Reader 19	Reader 20	Reader 21	ALL
1a+1b	1%	-16%	3%	9%	1%	3%	-4%	1%	10%	-1%	6%	-10%	-28%	2%	-7%	8%	-4%	5%	0%	4%	12%	0%

D — PERCENTAGE AGREEMENT BY EGG STAGE

	MODAL stage	Reader 1	Reader 2	Reader 3	Reader 4	Reader 5	Reader 6	Reader 7	Reader 8	Reader 9	Reader 10	Reader 11	Reader 12	Reader 13	Reader 14	Reader 15	Reader 16	Reader 17	Reader 18	Reader 19	Reader 20	Reader 21	ALL
Stage 1a ==>	0	97%	33%	97%	96%	93%	98%	66%	94%	53%	95%	98%	79%	88%	64%	88%	92%	53%	68%	54%	95%	57%	79%
Stage 1b ==>	1	0%	46%	18%	73%	78%	43%	63%	92%	51%	2%	2%	49%	2%	84%	76%	83%	27%	63%	96%	78%	53%	51%
Stage 2 ==>	2	83%	22%	75%	58%	81%	75%	47%	81%	32%	67%	72%	78%	81%	75%	81%	50%	81%	14%	81%	67%	33%	64%
Stage 3 ==>	3	77%	30%	74%	53%	65%	53%	74%	33%	35%	69%	59%	57%	67%	79%	51%	37%	63%	74%	73%	72%	69%	60%
Stage 4 ==>	4	72%	40%	28%	62%	58%	70%	55%	65%	35%	67%	71%	42%	68%	72%	57%	65%	48%	34%	71%	62%	26%	56%
Stage 5 ==>	5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Weighted mean	0-5	71.3%	34.9%	64.3%	74.7%	77.6%	74.0%	62.3%	77.1%	44.0%	67.0%	68.2%	63.3%	66.1%	72.1%	73.5%	71.9%	52.5%	54.9%	70.1%	78.9%	49.0%	65.1%
	RANKING	9	21	14	4	2	5	16	3	20	12	11	15	13	7	6	8	18	17	10	1	19	

E — PERCENTAGE AGREEMENT STAGE 1A and 1B combined

MODAL stage	Reader 1	Reader 2	Reader 3	Reader 4	Reader 5	Reader 6	Reader 7	Reader 8	Reader 9	Reader 10	Reader 11	Reader 12	Reader 13	Reader 14	Reader 15	Reader 16	Reader 17	Reader 18	Reader 19	Reader 20	Reader 21	ALL
1a+1b	96%	66%	97%	95%	92%	96%	85%	96%	88%	94%	97%	81%	66%	96%	89%	96%	91%	86%	96%	96%	95%	90%
RANKING	5	21	1	10	13	5	18	4	16	12	2	19	20	3	15	7	14	17	9	8	11	

F — BIAS

	MODAL stage	Reader 1	Reader 2	Reader 3	Reader 4	Reader 5	Reader 6	Reader 7	Reader 8	Reader 9	Reader 10	Reader 11	Reader 12	Reader 13	Reader 14	Reader 15	Reader 16	Reader 17	Reader 18	Reader 19	Reader 20	Reader 21	ALL
Stage 1a ==>	0	0.06	1.04	0.06	0.06	0.13	0.06	0.46	0.10	0.61	0.16	0.05	0.28	0.20	0.39	0.27	0.13	0.52	0.42	0.52	0.09	0.51	0.29
Stage 1b ==>	1	-0.88	0.21	-0.69	0.06	0.10	-0.37	0.33	0.08	-0.24	-0.78	-0.80	0.43	0.94	0.00	0.35	0.00	-0.27	0.24	0.08	-0.10	-0.35	-0.08
Stage 2 ==>	2	0.17	-0.33	0.08	-0.17	0.11	0.06	-0.06	0.22	-0.38	0.25	0.03	0.08	0.19	0.17	0.25	-0.25	0.14	-0.69	0.22	-0.08	-0.64	-0.03
Stage 3 ==>	3	-0.37	0.07	-0.33	-0.42	-0.28	-0.09	-0.19	0.12	-0.44	-0.36	-0.37	-0.67	-0.21	-0.30	-0.02	0.19	-0.35	-0.21	-0.05	-0.19	-0.29	-0.23
Stage 4 ==>	4	-0.28	0.10	0.12	-0.37	-0.37	-0.02	-0.43	0.00	-0.27	-0.33	-0.18	-0.35	-0.23	-0.28	0.12	0.17	-0.15	-0.17	-0.28	-0.13	-0.86	-0.20
Stage 5 ==>	5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Table 4.2.2. All eggs first staging, expert readers only.
(A) The numbers of eggs at each modal stage read by each participant. (B) The numbers of eggs allocated to each stage by each participant.
(C) The over / underestimation of stage 1 (1a+1b) by each participant. (D) The percentage agreement by modal egg stage by each participant.
(E) The percentage agreement by modal stage 1a and 1b combined, by each participant.
(F) The bias is indicated by the percentage over or underestimation of each egg stage, as estimated by each participant, in relation to the modal stage.
For each table the combined result is also given.

A

NUMBER OF EGG STAGE READINGS BY MODAL EGG STAGE

	MODAL stage	Reader 1	Reader 3	Reader 4	Reader 5	Reader 6	Reader 8	Reader 10	Reader 11	Reader 14	Reader 20	TOTAL
Stage 1a ==>	0	111	112	112	111	111	112	113	107	113	113	1115
Stage 1b ==>	1	47	47	47	47	47	47	47	44	47	47	467
Stage 2 ==>	2	35	35	35	35	35	35	35	35	35	35	350
Stage 3 ==>	3	45	45	45	45	45	45	44	43	45	45	447
Stage 4 ==>	4	58	58	58	57	58	58	58	54	58	58	575
Stage 5 ==>	5	-	-	-	-	-	-	-	-	-	-	-
Total	0-5	296	297	297	295	296	297	297	283	298	298	2954

B

EGG STAGE COMPOSITION

	Stage	Reader 1	Reader 3	Reader 4	Reader 5	Reader 6	Reader 8	Reader 10	Reader 11	Reader 14	Reader 20	TOTAL
Stage 1a ==>	0	158	152	129	117	140	109	157	158	83	125	1328
Stage 1b ==>	1	-	10	43	41	21	50	1	1	79	40	286
Stage 2 ==>	2	36	32	28	39	29	33	31	28	31	25	312
Stage 3 ==>	3	55	56	41	48	38	32	59	38	54	55	476
Stage 4 ==>	4	45	19	49	38	58	59	44	52	49	44	457
Stage 5 ==>	5	2	28	7	12	10	14	5	6	2	9	95
Total	0-5	296	297	297	295	296	297	297	283	298	298	2954

C

OVER- / UNDERESTIMATION OF STAGE 1 (=1A+1B)

	MODAL stage	Reader 1	Reader 3	Reader 4	Reader 5	Reader 6	Reader 8	Reader 10	Reader 11	Reader 14	Reader 20	ALL
	1a+1b	#WERT!	2%	8%	0%	2%	0%	-1%	5%	1%	3%	2%

D

PERCENTAGE AGREEMENT BY EGG STAGE

	MODAL stage	Reader 1	Reader 3	Reader 4	Reader 5	Reader 6	Reader 8	Reader 10	Reader 11	Reader 14	Reader 20	ALL
Stage 1a ==>	0	96%	97%	96%	90%	97%	91%	96%	98%	62%	93%	92%
Stage 1b ==>	1	0%	19%	77%	77%	45%	91%	2%	2%	83%	77%	48%
Stage 2 ==>	2	83%	77%	60%	80%	77%	83%	69%	74%	77%	69%	75%
Stage 3 ==>	3	78%	73%	51%	62%	53%	31%	68%	60%	76%	71%	62%
Stage 4 ==>	4	74%	28%	62%	60%	71%	66%	67%	74%	71%	62%	63%
Stage 5 ==>	5	-	-	-	-	-	-	-	-	-	-	-
Weighted mean	0-5	72.3%	65.3%	75.4%	76.6%	74.7%	76.1%	68.0%	70.0%	70.8%	78.2%	72.7%
	RANKING	6	10	4	2	5	3	9	8	7	1	

E

PERCENTAGE AGREEMENT STAGE 1A and 1B combined

	MODAL stage	Reader 1	Reader 3	Reader 4	Reader 5	Reader 6	Reader 8	Reader 10	Reader 11	Reader 14	Reader 20	ALL
	1a+1b	96%	97%	95%	92%	96%	96%	94%	97%	96%	96%	96%
	RANKING	5	1	8	10	5	4	9	2	3	7	

F

BIAS

	MODAL stage	Reader 1	Reader 3	Reader 4	Reader 5	Reader 6	Reader 8	Reader 10	Reader 11	Reader 14	Reader 20	ALL
Stage 1a ==>	0	0.08	0.05	0.05	0.16	0.07	0.14	0.16	0.05	0.42	0.13	0.13
Stage 1b ==>	1	-0.87	-0.68	0.11	0.11	-0.38	0.09	-0.77	-0.80	0.00	-0.11	-0.33
Stage 2 ==>	2	0.17	0.14	-0.11	0.11	0.11	0.20	0.31	0.09	0.14	-0.11	0.11
Stage 3 ==>	3	-0.36	-0.29	-0.44	-0.40	-0.07	0.18	-0.32	-0.35	-0.24	-0.16	-0.24
Stage 4 ==>	4	-0.26	0.14	-0.31	-0.25	0.00	-0.02	-0.33	-0.15	-0.29	-0.12	-0.16
Stage 5 ==>	5	-	-	-	-	-	-	-	-	-	-	-

Table 4.2.3. Mackerel eggs first staging.
(A) The numbers of eggs at each modal stage read by each participant. (B) The numbers of eggs allocated to each stage by each participant.
(C) The over / underestimation of stage 1 (1a+1b) by each participant. (D) The percentage agreement by modal egg stage by each participant.
(E) The percentage agreement by modal stage 1a and 1b combined, by each participant.
(F) The bias is indicated by the percentage over or underestimation of each egg stage, as estimated by each participant, in relation to the modal stage.
For each table the combined result is also given.

MACKEREL first staging Egg Stageing Workshop Bremerhaven, October 2018

A — NUMBER OF EGG STAGE READINGS BY MODAL EGG STAGE

	MODAL stage	Reader 1	Reader 2	Reader 3	Reader 4	Reader 5	Reader 6	Reader 7	Reader 8	Reader 9	Reader 10	Reader 11	Reader 12	Reader 13	Reader 14	Reader 15	Reader 16	Reader 17	Reader 18	Reader 19	Reader 20	Reader 21	TOTAL
Stage 1a ==>	0	50	27	62	59	50	53	44	63	54	61	58	53	53	58	61	56	24	48	69	49	22	1074
Stage 1b ==>	1	46	29	47	47	47	47	48	47	45	47	44	37	46	46	50	46	40	47	53	47	42	948
Stage 2 ==>	2	35	22	34	34	34	31	31	34	32	34	30	35	34	30	33	34	32	33	37	28	28	675
Stage 3 ==>	3	29	22	29	29	29	29	31	29	25	29	22	28	28	28	29	30	30	29	31	27	25	588
Stage 4 ==>	4	47	36	46	45	45	46	48	47	46	46	42	46	42	46	43	50	45	47	50	46	47	956
Stage 5 ==>	5	-	-	-	-	-	-	-	-	-	2	-	-	-	-	-	-	-	-	-	-	-	-
Total	0-5	207	136	218	214	205	206	202	220	202	219	196	199	203	208	216	216	171	204	240	197	164	4243

B — EGG STAGE COMPOSITION

	Stage	Reader 1	Reader 2	Reader 3	Reader 4	Reader 5	Reader 6	Reader 7	Reader 8	Reader 9	Reader 10	Reader 11	Reader 12	Reader 13	Reader 14	Reader 15	Reader 16	Reader 17	Reader 18	Reader 19	Reader 20	Reader 21	TOTAL
Stage 1a ==>	0	94	8	98	64	52	77	27	62	31	104	102	41	48	33	56	66	46	20	44	58	33	1164
Stage 1b ==>	1	0	36	10	42	38	21	52	45	64	1	1	29	5	69	43	44	16	76	76	38	39	745
Stage 2 ==>	2	29	28	26	27	32	25	33	29	29	29	23	52	73	26	33	18	27	23	33	20	17	632
Stage 3 ==>	3	47	10	49	35	44	26	50	28	29	47	23	42	38	41	29	15	41	46	40	40	52	772
Stage 4 ==>	4	36	38	7	39	31	47	35	48	27	33	41	23	34	38	36	55	29	22	47	33	20	719
Stage 5 ==>	5	1	16	28	7	8	10	5	8	22	5	6	12	5	1	19	18	12	17	-	8	3	211
Total	0-5	207	136	218	214	205	206	202	220	202	219	196	199	203	208	216	216	171	204	240	197	164	4243

C — OVER- / UNDERESTIMATION OF STAGE 1 (=1A+1B)

MODAL stage	Ned CvD Reader 1	Nor TdS Reader 2	Ned EB Reader 3	Sp PA Reader 4	Sp BB Reader 5	Id BOH Reader 6	Far SE Reader 7	Sp IR Reader 8	Sp GC Reader 9	Sco FB Reader 10	Sco JD Reader 11	Sp LI Reader 12	Por EH Reader 13	Ger TM Reader 14	Nor MF Reader 15	Nor AT Reader 16	Eng HLH Reader 17	Far DS Reader 18	Nor BM Reader 19	Den BH Reader 20	Den MKJ Reader 21	ALL
1a+1b	-2%	-21%	-1%	0%	-7%	-2%	-14%	-3%	-4%	-3%	1%	-22%	-46%	-2%	-11%	8%	-3%	1%	-2%	0%	13%	-6%

D — PERCENTAGE AGREEMENT BY EGG STAGE

	MODAL stage	Reader 1	Reader 2	Reader 3	Reader 4	Reader 5	Reader 6	Reader 7	Reader 8	Reader 9	Reader 10	Reader 11	Reader 12	Reader 13	Reader 14	Reader 15	Reader 16	Reader 17	Reader 18	Reader 19	Reader 20	Reader 21	ALL
Stage 1a ==>	0	98%	0%	97%	93%	94%	98%	52%	95%	20%	98%	98%	74%	85%	45%	87%	88%	79%	31%	64%	94%	41%	76%
Stage 1b ==>	1	0%	48%	19%	74%	77%	45%	63%	91%	49%	2%	2%	43%	0%	83%	74%	83%	28%	66%	94%	77%	57%	52%
Stage 2 ==>	2	83%	14%	76%	62%	79%	77%	52%	82%	31%	71%	73%	77%	82%	83%	79%	53%	78%	15%	81%	71%	39%	66%
Stage 3 ==>	3	93%	23%	97%	66%	86%	55%	94%	45%	40%	83%	59%	79%	79%	93%	69%	40%	80%	90%	81%	78%	84%	73%
Stage 4 ==>	4	72%	47%	15%	60%	60%	70%	65%	66%	33%	67%	71%	46%	67%	74%	51%	60%	56%	38%	80%	59%	32%	57%
Stage 5 ==>	5	-	-	-	-	-	-	-	-	-	100%	-	-	-	-	-	-	-	-	-	-	-	-
Weighted mean	0-5	67.1%	28.7%	59.6%	73.4%	75.0%	70.4%	63.9%	79.5%	33.7%	64.8%	62.8%	62.8%	60.6%	71.6%	73.1%	68.1%	60.8%	46.6%	78.8%	76.1%	48.8%	64.3%
RANKING		10	21	17	5	2	8	12	1	20	11	14	13	16	7	6	9	15	19	3	4	18	

E — PERCENTAGE AGREEMENT STAGE 1A and 1B combined

MODAL stage	Reader 1	Reader 2	Reader 3	Reader 4	Reader 5	Reader 6	Reader 7	Reader 8	Reader 9	Reader 10	Reader 11	Reader 12	Reader 13	Reader 14	Reader 15	Reader 16	Reader 17	Reader 18	Reader 19	Reader 20	Reader 21	ALL
1a+1b	97%	54%	97%	92%	92%	96%	78%	96%	82%	95%	96%	74%	53%	96%	89%	97%	95%	78%	98%	97%	94%	89%
RANKING	4	20	2	13	14	9	17	6	16	10	8	19	21	7	15	3	11	18	1	4	12	

F — BIAS

	MODAL stage	Reader 1	Reader 2	Reader 3	Reader 4	Reader 5	Reader 6	Reader 7	Reader 8	Reader 9	Reader 10	Reader 11	Reader 12	Reader 13	Reader 14	Reader 15	Reader 16	Reader 17	Reader 18	Reader 19	Reader 20	Reader 21	ALL
Stage 1a ==>	0	0.06	1.70	0.06	0.10	0.10	0.06	0.61	0.08	1.07	0.05	0.05	0.34	0.23	0.59	0.20	0.16	0.29	0.88	0.39	0.10	0.73	0.32
Stage 1b ==>	1	-0.87	0.10	-0.68	0.09	0.11	-0.38	0.33	0.09	-0.24	-0.77	-0.80	0.57	0.93	0.00	0.32	0.00	-0.58	0.30	0.09	-0.11	-0.29	-0.09
Stage 2 ==>	2	0.17	-0.23	0.15	-0.06	0.12	0.19	0.10	0.21	-0.25	0.29	0.07	0.09	0.18	0.13	0.30	-0.24	0.16	-0.58	0.22	0.07	-0.46	0.04
Stage 3 ==>	3	0.07	0.77	0.07	0.34	0.14	0.45	0.06	0.55	0.48	-0.03	0.23	-0.07	0.21	0.07	0.31	0.63	0.07	0.10	0.06	0.22	0.08	0.22
Stage 4 ==>	4	-0.30	0.28	0.26	-0.18	-0.04	0.07	-0.21	-0.06	0.17	-0.33	-0.07	-0.09	-0.17	-0.28	0.30	0.22	0.09	0.11	-0.24	-0.13	-0.64	-0.06
Stage 5 ==>	5	-	-	-	-	-	-	-	-	-	0.00	-	-	-	-	-	-	-	-	-	-	-	-

Table 4.2.4. Mackerel eggs first staging, expert readers only.
(A) The numbers of eggs at each modal stage read by each participant. (B) The numbers of eggs allocated to each stage by each participant.
(C) The over / underestimation of stage 1 (1a+1b) by each participant. (D) The percentage agreement by modal egg stage by each participant.
(E) The percentage agreement by modal stage 1a and 1b combined, by each participant.
(F) The bias is indicated by the percentage over or underestimation of each egg stage, as estimated by each participant, in relation to the modal stage.
For each table the combined result is also given.

A

NUMBER OF EGG STAGE READINGS BY MODAL EGG STAGE

	MODAL stage	Reader 1	Reader 3	Reader 4	Reader 5	Reader 6	Reader 8	Reader 10	Reader 11	Reader 14	Reader 20	TOTAL
Stage 1a ==>	0	50	62	60	50	53	63	61	58	59	49	565
Stage 1b ==>	1	46	47	46	47	47	47	47	44	45	47	463
Stage 2 ==>	2	35	34	34	34	31	34	34	30	30	28	324
Stage 3 ==>	3	30	30	30	30	30	30	29	23	29	27	288
Stage 4 ==>	4	46	45	44	44	45	46	46	41	45	46	448
Stage 5 ==>	5	-	-	-	-	-	-	2	-	-	-	-
Total	0-5	207	218	214	205	206	220	219	196	208	197	2090

B

EGG STAGE COMPOSITION

	Stage	Reader 1	Reader 3	Reader 4	Reader 5	Reader 6	Reader 8	Reader 10	Reader 11	Reader 14	Reader 20	TOTAL
Stage 1a ==>	0	94	98	64	52	77	62	104	102	33	58	744
Stage 1b ==>	1	0	10	42	38	21	45	1	1	69	38	265
Stage 2 ==>	2	29	26	27	32	25	29	29	23	26	20	266
Stage 3 ==>	3	47	49	35	44	26	28	47	23	41	40	380
Stage 4 ==>	4	36	7	39	31	47	48	33	41	38	33	353
Stage 5 ==>	5	1	28	7	8	10	8	5	6	1	8	82
Total	0-5	207	218	214	205	206	220	219	196	208	197	2090

C

OVER- / UNDERESTIMATION OF STAGE 1 (=1A+1B)

	MODAL stage	Reader 1	Reader 3	Reader 4	Reader 5	Reader 6	Reader 8	Reader 10	Reader 11	Reader 14	Reader 20	ALL
	1a+1b	-2%	-1%	0%	-7%	-2%	-3%	-3%	1%	-2%	0%	-2%

D

PERCENTAGE AGREEMENT BY EGG STAGE

	MODAL	Ned OvD	Ned EB	Sp PA	Sp BB	Irl BOH	Sp IR	Sco FB	Sco JD	Ger TM	Den BH	
	stage	Reader 1	Reader 3	Reader 4	Reader 5	Reader 6	Reader 8	Reader 10	Reader 11	Reader 14	Reader 20	ALL
Stage 1a ==>	0	98%	97%	93%	94%	98%	95%	98%	98%	46%	94%	91%
Stage 1b ==>	1	0%	19%	76%	77%	45%	91%	2%	2%	84%	77%	48%
Stage 2 ==>	2	83%	76%	62%	79%	77%	82%	71%	73%	83%	71%	76%
Stage 3 ==>	3	93%	93%	67%	87%	53%	47%	83%	57%	90%	78%	75%
Stage 4 ==>	4	74%	13%	61%	61%	69%	67%	67%	71%	73%	59%	61%
Stage 5 ==>	5	-	-	-	-	-	-	100%	-	-	-	-
Weighted mean	0-5	67.6%	59.2%	74.3%	79.5%	69.9%	80.0%	64.8%	62.2%	71.6%	76.1%	70.5%
	RANKING	7	10	4	2	6	1	8	9	5	3	

E

PERCENTAGE AGREEMENT STAGE 1A and 1B combined

	MODAL stage	Reader 1	Reader 3	Reader 4	Reader 5	Reader 6	Reader 8	Reader 10	Reader 11	Reader 14	Reader 20	ALL
	1a+1b	97%	97%	92%	92%	96%	96%	95%	96%	96%	97%	95%
	RANKING	2	1	9	10	7	4	8	6	5	2	

F

BIAS

	MODAL stage	Reader 1	Reader 3	Reader 4	Reader 5	Reader 6	Reader 8	Reader 10	Reader 11	Reader 14	Reader 20	ALL
Stage 1a ==>	0	0.06	0.06	0.10	0.10	0.06	0.08	0.05	0.05	0.58	0.10	0.13
Stage 1b ==>	1	-0.87	-0.68	0.11	0.11	-0.38	0.09	-0.77	-0.80	0.02	-0.11	-0.33
Stage 2 ==>	2	0.17	0.15	-0.06	0.12	0.19	0.21	0.29	0.07	0.13	0.07	0.14
Stage 3 ==>	3	0.07	0.10	0.33	0.13	0.47	0.53	-0.03	0.26	0.10	0.22	0.22
Stage 4 ==>	4	-0.28	0.27	-0.16	-0.02	0.07	-0.04	-0.33	-0.07	-0.29	-0.13	-0.10
Stage 5 ==>	5	-	-	-	-	-	-	0.00	-	-	-	-

Table 4.2.5. Horse mackerel eggs first staging.
(A) The numbers of eggs at each modal stage read by each participant. (B) The numbers of eggs allocated to each stage by each participant.
(C) The over / underestimation of stage 1 (1a+1b) by each participant. (D) The percentage agreement by modal egg stage by each participant.
(E) The percentage agreement by modal stage 1a and 1b combined, by each participant.
(F) The bias is indicated by the percentage over or underestimation of each egg stage, as estimated by each participant, in relation to the modal stage.
For each table the combined result is also given.

HORSE MACKEREL first staging Egg Stageing Workshop Bremerhaven, October 2018

A — NUMBER OF EGG STAGE READINGS BY MODAL EGG STAGE

	MODAL stage	Reader 1	Reader 2	Reader 3	Reader 4	Reader 5	Reader 6	Reader 7	Reader 8	Reader 9	Reader 10	Reader 11	Reader 12	Reader 13	Reader 14	Reader 15	Reader 16	Reader 17	Reader 18	Reader 19	Reader 20	Reader 21	TOTAL
Stage 1a ==>	0	35	40	34	33	34	37	31	27	23	29	35	38	42	36	32	36	31	35	34	33	25	700
Stage 1b ==>	1	-	19	-	-	-	-	5	-	10	-	1	14	1	3	-	-	39	-	-	2	29	0
Stage 2 ==>	2	1	8	-	-	-	-	5	-	8	-	2	5	-	2	2	-	6	1	-	-	4	44
Stage 3 ==>	3	-	7	-	-	-	-	3	-	5	-	4	1	2	2	1	-	5	4	-	1	5	-
Stage 4 ==>	4	-	7	-	-	-	-	1	-	4	-	3	-	-	-	2	-	-	1	-	-	2	-
Stage 5 ==>	5	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Total	0-5	36	82	34	33	34	37	45	27	50	29	45	58	45	43	37	36	81	41	34	36	65	928

B — EGG STAGE COMPOSITION

	Stage	Reader 1	Reader 2	Reader 3	Reader 4	Reader 5	Reader 6	Reader 7	Reader 8	Reader 9	Reader 10	Reader 11	Reader 12	Reader 13	Reader 14	Reader 15	Reader 16	Reader 17	Reader 18	Reader 19	Reader 20	Reader 21	TOTAL
Stage 1a ==>	0	36	34	34	33	34	37	30	27	24	27	35	36	42	36	32	36	30	34	13	33	27	670
Stage 1b ==>	1	-	17	-	-	-	-	8	-	15	-	0	12	1	3	-	-	39	-	21	2	26	144
Stage 2 ==>	2	-	15	-	-	-	-	-	-	2	1	3	10	-	2	2	-	9	1	-	-	4	49
Stage 3 ==>	3	-	9	-	-	-	-	6	-	6	1	4	-	2	2	-	-	3	3	-	1	6	43
Stage 4 ==>	4	-	5	-	-	-	-	1	-	3	-	3	-	-	-	3	-	-	3	-	-	2	20
Stage 5 ==>	5	-	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2
Total	0-5	36	82	34	33	34	37	45	27	50	29	45	58	45	43	37	36	81	41	34	36	65	928

C — OVER- / UNDERESTIMATION OF STAGE 1 (=1A+1B)

	MODAL stage	Reader 1	Reader 2	Reader 3	Reader 4	Reader 5	Reader 6	Reader 7	Reader 8	Reader 9	Reader 10	Reader 11	Reader 12	Reader 13	Reader 14	Reader 15	Reader 16	Reader 17	Reader 18	Reader 19	Reader 20	Reader 21	ALL
	1a+1b	3%	-14%	0%	0%	0%	0%	6%	0%	18%	-7%	-3%	-8%	0%	0%	0%	0%	-1%	-3%	-62%	0%	-2%	16%

D — PERCENTAGE AGREEMENT BY EGG STAGE

	MODAL stage	Reader 1	Reader 2	Reader 3	Reader 4	Reader 5	Reader 6	Reader 7	Reader 8	Reader 9	Reader 10	Reader 11	Reader 12	Reader 13	Reader 14	Reader 15	Reader 16	Reader 17	Reader 18	Reader 19	Reader 20	Reader 21	ALL
Stage 1a ==>	0	100%	70%	100%	100%	100%	100%	84%	100%	91%	93%	100%	84%	100%	100%	100%	100%	94%	97%	38%	100%	84%	92%
Stage 1b ==>	1	-	47%	-	-	-	-	60%	-	90%	-	0%	64%	100%	100%	-	-	97%	-	-	100%	79%	-
Stage 2 ==>	2	0%	50%	-	-	-	-	0%	-	25%	-	100%	100%	-	100%	100%	-	100%	100%	-	-	100%	72%
Stage 3 ==>	3	-	71%	-	-	-	-	67%	-	80%	-	100%	0%	100%	100%	0%	-	60%	75%	-	100%	100%	-
Stage 4 ==>	4	-	57%	-	-	-	-	0%	-	50%	-	100%	-	-	-	100%	-	-	100%	-	-	100%	-
Stage 5 ==>	5	-	100%	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Weighted mean	0-5	97.2%	62.2%	100.0%	100.0%	100.0%	100.0%	68.9%	100.0%	76.0%	93.1%	97.8%	79.3%	100.0%	100.0%	97.3%	100.0%	93.8%	95.1%	38.2%	100.0%	84.6%	87.9%
	RANKING	12	20	1	1	1	1	19	1	18	15	10	17	1	1	11	1	14	13	21	1	16	

E — PERCENTAGE AGREEMENT STAGE 1A and 1B combined

	MODAL stage	Reader 1	Reader 2	Reader 3	Reader 4	Reader 5	Reader 6	Reader 7	Reader 8	Reader 9	Reader 10	Reader 11	Reader 12	Reader 13	Reader 14	Reader 15	Reader 16	Reader 17	Reader 18	Reader 19	Reader 20	Reader 21	ALL
	1a+1b	100%	83%	100%	100%	100%	100%	97%	100%	100%	93%	97%	92%	100%	100%	100%	100%	99%	97%	100%	100%	98%	97%
	RANKING	1	21	1	1	1	1	16	1	1	19	16	20	1	1	1	1	14	18	1	1	15	

F — BIAS

	MODAL stage	Reader 1	Reader 2	Reader 3	Reader 4	Reader 5	Reader 6	Reader 7	Reader 8	Reader 9	Reader 10	Reader 11	Reader 12	Reader 13	Reader 14	Reader 15	Reader 16	Reader 17	Reader 18	Reader 19	Reader 20	Reader 21	ALL
Stage 1a ==>	0	0.00	0.43	0.00	0.00	0.00	0.00	0.23	0.00	0.09	0.17	0.00	0.24	0.00	0.00	0.00	0.00	0.10	0.11	0.62	0.00	0.24	0.11
Stage 1b ==>	1	-	0.00	-	-	-	-	-0.40	-	-0.10	-	1.00	-0.21	0.00	0.00	-	-	-0.03	-	-	0.00	-0.21	-0.10
Stage 2 ==>	2	-2.00	-0.13	-	-	-	-	0.20	-	-1.00	-	0.00	0.00	-	0.00	0.00	-	0.00	0.00	-	-	0.00	-0.23
Stage 3 ==>	3	-	0.00	-	-	-	-	-1.00	-	0.20	-	0.00	-1.00	0.00	0.00	1.00	-	-0.40	0.25	-	0.00	0.00	-
Stage 4 ==>	4	-	-0.14	-	-	-	-	-1.00	-	-0.50	-	0.00	-	-	-	0.00	-	-	0.00	-	-	0.00	-
Stage 5 ==>	5	-	0.00	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Table 4.2.6. Horse mackerel eggs first staging, expert readers only.
(A) The numbers of eggs at each modal stage read by each participant. (B) The numbers of eggs allocated to each stage by each participant.
(C) The over / underestimation of stage 1 (1a+1b) by each participant. (D) The percentage agreement by modal egg stage by each participant.
(E) The percentage agreement by modal stage 1a and 1b combined, by each participant.
(F) The bias is indicated by the percentage over or underestimation of each egg stage, as estimated by each participant, in relation to the modal stage.
For each table the combined result is also given.

A

NUMBER OF EGG STAGE READINGS BY MODAL EGG STAGE

	MODAL stage	Reader 1	Reader 3	Reader 4	Reader 5	Reader 6	Reader 8	Reader 10	Reader 11	Reader 14	Reader 20	TOTAL
Stage 1a ==>	0	36	34	33	34	37	27	29	35	36	33	334
Stage 1b ==>	1	-	-	-	-	-	-	-	-	3	2	0
Stage 2 ==>	2	-	-	-	-	-	-	-	3	2	-	-
Stage 3 ==>	3	-	-	-	-	-	-	-	4	2	1	-
Stage 4 ==>	4	-	-	-	-	-	-	-	3	-	-	-
Stage 5 ==>	5	-	-	-	-	-	-	-	-	-	-	-
Total	0-5	36	34	33	34	37	27	29	45	43	36	354

B

EGG STAGE COMPOSITION

	Stage	Reader 1	Reader 3	Reader 4	Reader 5	Reader 6	Reader 8	Reader 10	Reader 11	Reader 14	Reader 20	TOTAL
Stage 1a ==>	0	36	34	33	34	37	27	27	35	36	33	332
Stage 1b ==>	1	-	-	-	-	-	-	-	0	3	2	5
Stage 2 ==>	2	-	-	-	-	-	-	1	3	2	-	6
Stage 3 ==>	3	-	-	-	-	-	-	1	4	2	1	8
Stage 4 ==>	4	-	-	-	-	-	-	-	3	-	-	3
Stage 5 ==>	5	-	-	-	-	-	-	-	-	-	-	-
Total	0-5	36	34	33	34	37	27	29	45	43	36	354

C

OVER- / UNDERESTIMATION OF STAGE 1 (=1A+1B)

MODAL stage	Reader 1	Reader 3	Reader 4	Reader 5	Reader 6	Reader 8	Reader 10	Reader 11	Reader 14	Reader 20	ALL
1a+1b	0%	0%	0%	0%	0%	0%	-7%	0%	0%	0%	1%

D

PERCENTAGE AGREEMENT BY EGG STAGE

	MODAL stage	Reader 1	Reader 3	Reader 4	Reader 5	Reader 6	Reader 8	Reader 10	Reader 11	Reader 14	Reader 20	ALL
Stage 1a ==>	0	100%	100%	100%	100%	100%	100%	93%	100%	100%	100%	99%
Stage 1b ==>	1	-	-	-	-	-	-	-	-	100%	100%	-
Stage 2 ==>	2	-	-	-	-	-	-	-	100%	100%		-
Stage 3 ==>	3	-	-	-	-	-	-	-	100%	100%	100%	-
Stage 4 ==>	4	-	-	-	-	-	-	-	100%	-	-	-
Stage 5 ==>	5	-	-	-	-	-	-	-	-	-	-	-
Weighted mean	0-5	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%	93.1%	100.0%	100.0%	100.0%	99.4%
RANKING		1	1	1	1	1	1	10	1	1	1	

E

PERCENTAGE AGREEMENT STAGE 1A and 1B combined

MODAL stage	Reader 1	Reader 3	Reader 4	Reader 5	Reader 6	Reader 8	Reader 10	Reader 11	Reader 14	Reader 20	ALL
1a+1b	100%	100%	100%	100%	100%	100%	93%	100%	100%	100%	99%
RANKING	1	1	1	1	1	1	10	1	1	1	

F

BIAS

	MODAL stage	Reader 1	Reader 3	Reader 4	Reader 5	Reader 6	Reader 8	Reader 10	Reader 11	Reader 14	Reader 20	ALL
Stage 1a ==>	0	0.00	0.00	0.00	0.00	0.00	0.00	0.17	0.00	0.00	0.00	0.01
Stage 1b ==>	1	-	-	-	-	-	-	-	-	0.00	0.00	0.00
Stage 2 ==>	2	-	-	-	-	-	-	-	0.00	0.00	-	-
Stage 3 ==>	3	-	-	-	-	-	-	-	0.00	0.00	0.00	-
Stage 4 ==>	4	-	-	-	-	-	-	-	0.00	-	-	-
Stage 5 ==>	5	-	-	-	-	-	-	-	-	-	-	-

Table 4.2.7. All eggs second staging.(A) The numbers of eggs at each modal stage read by each participant. (B) The numbers of eggs allocated to each stage by each participant. (C) The over / underestimation of stage 1 (1a+1b) by each participant. (D) The percentage agreement by modal egg stage by each participant. (E) The percentage agreement by modal stage 1a and 1b combined, by each participant. (F) The bias is indicated by the percentage over or underestimation of each egg stage, as estimated by each participant, in relation to the modal stage. For each table the combined result is also given.

ALL EGGS second stageing Egg Stageing Workshop Bremerhaven, October 2018

A

NUMBER OF EGG STAGE READINGS BY MODAL EGG STAGE

	MODAL stage	Reader 1	Reader 2	Reader 3	Reader 4	Reader 5	Reader 6	Reader 7	Reader 8	Reader 9	Reader 10	Reader 11	Reader 12	Reader 13	Reader 14	Reader 15	Reader 16	Reader 17	Reader 18	Reader 19	TOTAL
Stage 1a ==>	0	86	84	86	86	86	86	86	86	86	86	86	86	86	86	86	86	85	86	86	1631
Stage 1b ==>	1	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	475
Stage 2 ==>	2	39	39	39	39	39	39	39	39	39	39	39	39	39	39	39	39	38	39	39	740
Stage 3 ==>	3	55	55	56	56	56	56	56	55	56	55	56	56	56	56	56	56	56	56	55	1059
Stage 4 ==>	4	24	24	24	24	24	24	24	24	23	24	24	24	24	24	24	24	24	24	24	455
Stage 5 ==>	5	8	8	7	8	8	8	8	7	8	8	8	8	8	8	8	8	8	8	8	150
Total	0-5	237	235	237	238	238	238	238	236	237	237	238	238	238	238	238	238	236	238	237	4510

B

EGG STAGE COMPOSITION

	Stage	Reader 1	Reader 2	Reader 3	Reader 4	Reader 5	Reader 6	Reader 7	Reader 8	Reader 9	Reader 10	Reader 11	Reader 12	Reader 13	Reader 14	Reader 15	Reader 16	Reader 17	Reader 18	Reader 19	TOTAL
Stage 1a ==>	0	84	95	85	86	93	66	86	95	93	72	82	113	89	77	82	67	39	78	90	1572
Stage 1b ==>	1	31	18	26	27	19	48	28	16	20	44	28	3	21	38	31	51	74	33	22	578
Stage 2 ==>	2	58	51	50	40	34	31	29	53	48	52	32	33	47	27	37	31	33	28	29	743
Stage 3 ==>	3	51	51	38	58	55	70	52	50	42	45	64	59	51	72	24	39	15	78	73	987
Stage 4 ==>	4	9	14	36	20	33	21	36	19	30	15	27	27	29	23	62	31	74	16	21	543
Stage 5 ==>	5	4	6	2	7	4	2	7	3	4	9	5	3	1	1	2	19	1	5	2	87
Total	0-5	237	235	237	238	238	238	238	236	237	237	238	238	238	238	238	238	236	238	237	4510

C

OVER- / UNDERESTIMATION OF STAGE 1 (=1A+1B)

	MODAL stage	Reader 1	Reader 2	Reader 3	Reader 4	Reader 5	Reader 6	Reader 7	Reader 8	Reader 9	Reader 10	Reader 11	Reader 12	Reader 13	Reader 14	Reader 15	Reader 16	Reader 17	Reader 18	Reader 19	ALL
	1a+1b	4%	4%	0%	2%	1%	3%	3%	0%	2%	5%	-1%	5%	-1%	4%	2%	6%	3%	0%	1%	2%

D

PERCENTAGE AGREEMENT BY EGG STAGE

	MODAL stage	Reader 1	Reader 2	Reader 3	Reader 4	Reader 5	Reader 6	Reader 7	Reader 8	Reader 9	Reader 10	Reader 11	Reader 12	Reader 13	Reader 14	Reader 15	Reader 16	Reader 17	Reader 18	Reader 19	ALL
Stage 1a ==>	0	90%	96%	94%	95%	100%	74%	99%	98%	99%	72%	92%	98%	98%	88%	93%	77%	46%	90%	97%	89%
Stage 1b ==>	1	84%	64%	84%	100%	76%	96%	100%	60%	76%	80%	92%	4%	80%	96%	92%	100%	100%	100%	84%	83%
Stage 2 ==>	2	87%	87%	97%	95%	82%	64%	72%	97%	92%	79%	74%	82%	90%	64%	87%	67%	79%	69%	64%	81%
Stage 3 ==>	3	56%	67%	61%	84%	82%	91%	73%	73%	64%	53%	86%	88%	70%	93%	36%	61%	20%	100%	89%	71%
Stage 4 ==>	4	33%	33%	88%	50%	88%	58%	71%	58%	74%	33%	67%	63%	63%	54%	92%	46%	100%	50%	50%	62%
Stage 5 ==>	5	50%	38%	29%	50%	50%	25%	50%	43%	50%	38%	50%	25%	13%	13%	25%	88%	13%	50%	13%	37%
Weighted mean	0-5	73.8%	76.2%	83.1%	87.0%	87.4%	75.6%	84.0%	82.2%	83.1%	64.6%	83.6%	76.9%	81.5%	80.3%	76.1%	71.0%	55.1%	84.5%	80.6%	78.2%
	RANKING	16	13	6	2	1	15	4	8	6	18	5	12	9	11	14	17	19	3	10	

E

PERCENTAGE AGREEMENT STAGE 1A and 1B combined

	MODAL stage	Reader 1	Reader 2	Reader 3	Reader 4	Reader 5	Reader 6	Reader 7	Reader 8	Reader 9	Reader 10	Reader 11	Reader 12	Reader 13	Reader 14	Reader 15	Reader 16	Reader 17	Reader 18	Reader 19	ALL
	1a+1b	98%	99%	100%	98%	100%	98%	99%	99%	99%	97%	97%	100%	97%	98%	100%	98%	100%	99%	98%	99%
	RANKING	11	10	1	11	1	11	6	6	6	17	17	1	17	11	1	11	1	6	11	

F

BIAS

	MODAL stage	Reader 1	Reader 2	Reader 3	Reader 4	Reader 5	Reader 6	Reader 7	Reader 8	Reader 9	Reader 10	Reader 11	Reader 12	Reader 13	Reader 14	Reader 15	Reader 16	Reader 17	Reader 18	Reader 19	ALL
Stage 1a ==>	0	0.15	0.05	0.06	0.07	0.00	0.28	0.02	0.03	0.02	0.34	0.10	0.02	0.05	0.14	0.07	0.26	0.54	0.12	0.07	0.13
Stage 1b ==>	1	-0.16	-0.36	-0.16	0.00	-0.24	-0.04	0.00	-0.40	-0.24	-0.20	0.04	-0.96	-0.12	0.12	-0.08	0.00	0.00	0.00	-0.16	-0.16
Stage 2 ==>	2	-0.13	-0.10	0.03	0.05	0.10	0.08	0.05	-0.05	-0.13	-0.10	0.10	-0.21	0.05	0.10	0.08	-0.13	0.08	0.23	0.13	0.01
Stage 3 ==>	3	-0.49	-0.29	-0.04	0.07	0.11	-0.02	0.27	-0.20	0.04	-0.33	0.11	0.09	-0.09	-0.05	0.54	0.30	0.70	0.00	0.00	0.03
Stage 4 ==>	4	-0.75	-0.46	-0.13	-0.46	-0.13	-0.46	-0.04	-0.42	-0.26	-0.50	-0.25	-0.29	-0.38	-0.46	-0.08	0.38	0.00	-0.42	-0.42	-0.29
Stage 5 ==>	5	-0.88	-1.13	-0.71	-0.50	-0.50	-0.88	-0.50	-0.86	-0.50	-0.75	-0.50	-0.75	-0.88	-0.88	-0.88	-0.13	-0.88	-0.50	-1.00	-0.71

Table 4.2.8. All eggs second staging, expert readers only.
(A) The numbers of eggs at each modal stage read by each participant. (B) The numbers of eggs allocated to each stage by each participant.
(C) The over / underestimation of stage 1 (1a+1b) by each participant. (D) The percentage agreement by modal egg stage by each participant.
(E) The percentage agreement by modal stage 1a and 1b combined, by each participant.
(F) The bias is indicated by the percentage over or underestimation of each egg stage, as estimated by each participant, in relation to the modal stage.
For each table the combined result is also given.

A

NUMBER OF EGG STAGE READINGS BY MODAL EGG STAGE

	MODAL stage	Reader 2	Reader 3	Reader 4	Reader 5	Reader 7	Reader 8	Reader 9	Reader 12	Reader 18	TOTAL
Stage 1a ==>	0	84	86	86	86	86	86	86	86	86	772
Stage 1b ==>	1	25	25	25	25	25	25	25	25	25	225
Stage 2 ==>	2	39	39	39	39	39	39	39	39	39	351
Stage 3 ==>	3	54	55	55	55	55	54	55	55	55	493
Stage 4 ==>	4	25	25	25	25	25	25	24	25	25	224
Stage 5 ==>	5	8	7	8	8	8	7	8	8	8	70
Total	0-5	235	237	238	238	238	236	237	238	238	2135

B

EGG STAGE COMPOSITION

	Stage	Reader 2	Reader 3	Reader 4	Reader 5	Reader 7	Reader 8	Reader 9	Reader 12	Reader 18	TOTAL
Stage 1a ==>	0	95	85	86	93	86	95	93	113	78	824
Stage 1b ==>	1	18	26	27	19	28	16	20	3	33	190
Stage 2 ==>	2	51	50	40	34	29	53	48	33	28	366
Stage 3 ==>	3	51	38	58	55	52	50	42	59	78	483
Stage 4 ==>	4	14	36	20	33	36	19	30	27	16	231
Stage 5 ==>	5	6	2	7	4	7	3	4	3	5	41
Total	0-5	235	237	238	238	238	236	237	238	238	2135

C

OVER- / UNDERESTIMATION OF STAGE 1 (=1A+1B)

	MODAL stage	Reader 2	Reader 3	Reader 4	Reader 5	Reader 7	Reader 8	Reader 9	Reader 12	Reader 18	ALL
	1a+1b	4%	0%	2%	1%	3%	0%	2%	5%	0%	2%

D

PERCENTAGE AGREEMENT BY EGG STAGE

	MODAL stage	Reader 2	Reader 3	Reader 4	Reader 5	Reader 7	Reader 8	Reader 9	Reader 12	Reader 18	ALL
Stage 1a ==>	0	96%	94%	95%	100%	99%	98%	99%	98%	90%	97%
Stage 1b ==>	1	64%	84%	100%	76%	100%	60%	76%	4%	100%	74%
Stage 2 ==>	2	87%	100%	97%	82%	72%	97%	95%	82%	69%	87%
Stage 3 ==>	3	69%	64%	87%	82%	75%	74%	64%	89%	100%	78%
Stage 4 ==>	4	36%	88%	52%	84%	72%	60%	71%	64%	48%	64%
Stage 5 ==>	5	38%	29%	50%	50%	50%	43%	50%	25%	50%	43%
Weighted mean	0-5	76.6%	84.4%	88.2%	87.0%	84.5%	82.6%	83.1%	77.3%	84.0%	83.1%
RANKING		9	4	1	2	3	7	6	8	5	

E

PERCENTAGE AGREEMENT STAGE 1A and 1B combined

	MODAL stage	Reader 2	Reader 3	Reader 4	Reader 5	Reader 7	Reader 8	Reader 9	Reader 12	Reader 18	ALL
	1a+1b	99%	100%	98%	100%	99%	99%	99%	100%	99%	99%
RANKING		8	1	9	1	4	4	4	1	4	

F

BIAS

	MODAL stage	Reader 2	Reader 3	Reader 4	Reader 5	Reader 7	Reader 8	Reader 9	Reader 12	Reader 18	ALL
Stage 1a ==>	0	0.05	0.06	0.07	0.00	0.02	0.03	0.02	0.02	0.12	0.04
Stage 1b ==>	1	-0.36	-0.16	0.00	-0.24	0.00	-0.40	-0.24	-0.96	0.00	-0.26
Stage 2 ==>	2	-0.10	0.00	0.03	0.10	0.05	-0.05	-0.08	-0.21	0.23	-0.00
Stage 3 ==>	3	-0.31	-0.04	-0.07	0.11	0.25	-0.22	-0.07	0.07	0.00	-0.03
Stage 4 ==>	4	-0.44	-0.12	-0.44	-0.16	-0.04	-0.40	-0.29	-0.28	-0.44	-0.29
Stage 5 ==>	5	-1.13	-0.71	-0.50	-0.50	-0.50	-0.86	-0.50	-0.75	-0.50	-0.66

Table 4.2.9. Mackerel eggs second staging.(A) The numbers of eggs at each modal stage read by each participant. (B) The numbers of eggs allocated to each stage by each participant.(C) The over / underestimation of stage 1 (1a+1b) by each participant. (D) The percentage agreement by modal egg stage by each participant. (E) The percentage agreement by modal stage 1a and 1b combined, by each participant. (F) The bias is indicated by the percentage over or underestimation of each egg stage, as estimated by each participant, in relation to the modal stage. For each table the combined result is also given.

MACKEREL second stageing Egg Stageing Workshop Bremerhaven, October 2018

A

NUMBER OF EGG STAGE READINGS BY MODAL EGG STAGE

	MODAL stage	Reader 1	Reader 2	Reader 3	Reader 4	Reader 5	Reader 6	Reader 7	Reader 8	Reader 9	Reader 10	Reader 11	Reader 12	Reader 13	Reader 14	Reader 15	Reader 16	Reader 17	Reader 18	Reader 19	TOTAL
Stage 1a ⟹	0	39	37	46	37	33	34	38	37	37	44	33	48	39	41	35	34	38	33	35	718
Stage 1b ⟹	1	25	25	23	25	24	23	25	25	26	25	24	25	25	25	23	26	25	25	25	469
Stage 2 ⟹	2	25	21	23	22	21	24	20	20	20	19	19	24	21	21	19	21	19	20	25	404
Stage 3 ⟹	3	37	34	33	34	32	34	33	32	35	34	32	33	31	33	34	34	34	31	43	643
Stage 4 ⟹	4	20	20	20	20	20	20	20	20	20	20	20	20	19	20	19	20	20	19	19	376
Stage 5 ⟹	5	8	8	8	8	7	7	8	7	8	7	8	7	6	8	7	8	7	6	8	141
Total	0-5	154	145	153	146	137	142	144	141	146	149	136	157	141	148	137	143	143	134	155	2751

B

EGG STAGE COMPOSITION

	Stage	Reader 1	Reader 2	Reader 3	Reader 4	Reader 5	Reader 6	Reader 7	Reader 8	Reader 9	Reader 10	Reader 11	Reader 12	Reader 13	Reader 14	Reader 15	Reader 16	Reader 17	Reader 18	Reader 19	TOTAL
Stage 1a ⟹	0	42	45	47	36	38	33	38	47	45	32	35	75	43	39	31	30	33	31	40	760
Stage 1b ⟹	1	25	18	22	26	19	26	25	15	20	38	24	1	21	29	27	34	31	27	22	450
Stage 2 ⟹	2	34	24	25	22	20	21	19	23	20	30	19	21	23	17	21	16	16	19	21	411
Stage 3 ⟹	3	41	41	26	39	28	43	27	38	30	32	31	37	34	45	11	20	5	42	54	624
Stage 4 ⟹	4	8	12	31	17	28	17	28	15	27	10	22	20	19	17	45	26	58	10	17	427
Stage 5 ⟹	5	4	5	2	6	4	2	7	3	4	7	5	3	1	1	2	17	-	5	1	79
Total	0-5	154	145	153	146	137	142	144	141	146	149	136	157	141	148	137	143	143	134	155	2751

C

OVER- / UNDERESTIMATION OF STAGE 1 (=1A+1B)

MODAL stage	Reader 1	Reader 2	Reader 3	Reader 4	Reader 5	Reader 6	Reader 7	Reader 8	Reader 9	Reader 10	Reader 11	Reader 12	Reader 13	Reader 14	Reader 15	Reader 16	Reader 17	Reader 18	Reader 19	ALL
1a+1b	5%	2%	0%	0%	0%	4%	0%	0%	3%	1%	4%	4%	0%	3%	0%	7%	2%	0%	3%	2%

D

PERCENTAGE AGREEMENT BY EGG STAGE

	MODAL stage	Reader 1	Reader 2	Reader 3	Reader 4	Reader 5	Reader 6	Reader 7	Reader 8	Reader 9	Reader 10	Reader 11	Reader 12	Reader 13	Reader 14	Reader 15	Reader 16	Reader 17	Reader 18	Reader 19	ALL
Stage 1a ⟹	0	95%	92%	93%	97%	100%	94%	100%	100%	100%	59%	97%	100%	100%	95%	89%	88%	87%	94%	97%	93%
Stage 1b ⟹	1	84%	64%	83%	100%	79%	96%	100%	60%	73%	80%	96%	4%	80%	96%	100%	96%	100%	100%	84%	83%
Stage 2 ⟹	2	84%	95%	100%	100%	95%	88%	95%	100%	90%	89%	89%	88%	95%	81%	100%	71%	84%	95%	84%	90%
Stage 3 ⟹	3	70%	91%	70%	91%	75%	97%	67%	91%	69%	62%	78%	88%	81%	100%	29%	50%	9%	100%	95%	74%
Stage 4 ⟹	4	35%	40%	85%	50%	85%	55%	65%	55%	70%	30%	65%	55%	53%	45%	95%	45%	100%	42%	47%	59%
Stage 5 ⟹	5	50%	38%	25%	50%	57%	29%	50%	43%	50%	57%	50%	29%	17%	13%	29%	88%	0%	67%	13%	40%
Weighted mean	0-5	75.3%	77.2%	83.0%	87.7%	85.4%	85.2%	84.0%	81.6%	79.5%	63.1%	83.8%	71.3%	81.6%	83.1%	75.2%	72.0%	67.8%	88.1%	81.9%	79.2%
RANKING		14	13	8	2	3	4	5	10	12	19	6	17	10	7	15	16	18	1	9	

E

PERCENTAGE AGREEMENT STAGE 1A and 1B combined

MODAL stage	Reader 1	Reader 2	Reader 3	Reader 4	Reader 5	Reader 6	Reader 7	Reader 8	Reader 9	Reader 10	Reader 11	Reader 12	Reader 13	Reader 14	Reader 15	Reader 16	Reader 17	Reader 18	Reader 19	ALL
1a+1b	100%	98%	100%	100%	100%	100%	100%	100%	100%	97%	100%	100%	98%	98%	100%	100%	100%	100%	100%	100%
RANKING	1	18	1	1	1	1	1	1	1	19	1	1	17	16	1	1	1	1	1	

F

BIAS

	MODAL stage	Reader 1	Reader 2	Reader 3	Reader 4	Reader 5	Reader 6	Reader 7	Reader 8	Reader 9	Reader 10	Reader 11	Reader 12	Reader 13	Reader 14	Reader 15	Reader 16	Reader 17	Reader 18	Reader 19	ALL
Stage 1a ⟹	0	0.05	0.11	0.07	0.03	0.00	0.06	0.00	0.00	0.00	0.48	0.03	0.00	0.00	0.05	0.11	0.12	0.13	0.06	0.03	0.07
Stage 1b ⟹	1	-0.16	-0.36	-0.17	0.00	-0.21	-0.04	0.00	-0.40	-0.27	-0.20	-0.04	-0.96	-0.12	0.12	0.00	0.04	0.00	0.00	-0.16	-0.16
Stage 2 ⟹	2	-0.12	-0.10	0.00	0.00	0.05	-0.04	0.05	0.00	-0.15	-0.11	-0.21	-0.25	-0.05	-0.10	0.00	-0.19	0.05	0.05	-0.08	-0.06
Stage 3 ⟹	3	0.30	0.09	0.18	0.09	0.25	0.03	0.33	-0.03	0.20	-0.32	0.09	0.12	0.06	0.00	0.59	0.53	0.91	0.00	0.05	0.14
Stage 4 ⟹	4	-0.75	-0.40	-0.15	-0.30	-0.15	-0.45	-0.05	-0.45	-0.30	-0.55	-0.25	-0.35	-0.47	-0.55	-0.05	0.35	0.00	-0.47	-0.53	-0.31
Stage 5 ⟹	5	-0.88	-1.13	-0.75	-0.50	-0.43	-0.71	-0.50	-0.86	-0.50	-0.43	-0.50	-0.71	-0.83	-0.88	-0.71	-0.13	-1.00	-0.33	-1.00	-0.67

Table 4.2.10. Mackerel eggs second staging, expert readers only.
(A) The numbers of eggs at each modal stage read by each participant. (B) The numbers of eggs allocated to each stage by each participant.
(C) The over / underestimation of stage 1 (1a+1b) by each participant. (D) The percentage agreement by modal egg stage by each participant.
(E) The percentage agreement by modal stage 1a and 1b combined, by each participant.
(F) The bias is indicated by the percentage over or underestimation of each egg stage, as estimated by each participant, in relation to the modal stage.
For each table the combined result is also given.

A

NUMBER OF EGG STAGE READINGS BY MODAL EGG STAGE

	MODAL stage	Reader 2	Reader 3	Reader 4	Reader 5	Reader 7	Reader 8	Reader 9	Reader 12	Reader 18	TOTAL
Stage 1a ==>	0	37	46	37	33	38	37	38	48	33	347
Stage 1b ==>	1	25	23	25	24	25	25	25	25	25	222
Stage 2 ==>	2	21	23	22	21	19	20	20	23	20	189
Stage 3 ==>	3	34	33	34	32	34	32	35	34	31	299
Stage 4 ==>	4	20	21	20	20	20	20	20	20	19	180
Stage 5 ==>	5	8	7	8	7	8	7	8	7	6	66
Total	0-5	145	153	146	137	144	141	146	157	134	1303

B

EGG STAGE COMPOSITION

	Stage	Reader 2	Reader 3	Reader 4	Reader 5	Reader 7	Reader 8	Reader 9	Reader 12	Reader 18	TOTAL
Stage 1a ==>	0	45	47	36	38	38	47	45	75	31	402
Stage 1b ==>	1	18	22	26	19	25	15	20	1	27	173
Stage 2 ==>	2	24	25	22	20	19	23	20	21	19	193
Stage 3 ==>	3	41	26	39	28	27	38	30	37	42	308
Stage 4 ==>	4	12	31	17	28	28	15	27	20	10	188
Stage 5 ==>	5	5	2	6	4	7	3	4	3	5	39
Total	0-5	145	153	146	137	144	141	146	157	134	1303

C

OVER- / UNDERESTIMATION OF STAGE 1 (=1A+1B)

MODAL stage	Reader 2	Reader 3	Reader 4	Reader 5	Reader 7	Reader 8	Reader 9	Reader 12	Reader 18	ALL
1a+1b	2%	0%	0%	0%	0%	0%	3%	4%	0%	1%

D

PERCENTAGE AGREEMENT BY EGG STAGE

	MODAL stage	Reader 2	Reader 3	Reader 4	Reader 5	Reader 7	Reader 8	Reader 9	Reader 12	Reader 18	ALL
Stage 1a ==>	0	92%	93%	97%	100%	100%	100%	100%	100%	94%	97%
Stage 1b ==>	1	64%	83%	100%	79%	100%	60%	76%	4%	100%	74%
Stage 2 ==>	2	95%	100%	100%	95%	100%	100%	90%	87%	95%	96%
Stage 3 ==>	3	91%	70%	91%	75%	68%	91%	69%	85%	100%	82%
Stage 4 ==>	4	40%	86%	50%	85%	65%	55%	70%	55%	42%	61%
Stage 5 ==>	5	38%	29%	50%	57%	50%	43%	50%	29%	67%	45%
Weighted mean	0-5	77.2%	83.7%	87.7%	85.4%	84.7%	81.6%	80.1%	70.7%	88.1%	82.0%
RANKING		8	5	2	3	4	6	7	9	1	

E

PERCENTAGE AGREEMENT STAGE 1A and 1B combined

MODAL stage	Reader 2	Reader 3	Reader 4	Reader 5	Reader 7	Reader 8	Reader 9	Reader 12	Reader 18	ALL
1a+1b	98%	100%	100%	100%	100%	100%	100%	100%	100%	100%
RANKING	9	1	1	1	1	1	1	1	1	

F

BIAS

	MODAL stage	Reader 2	Reader 3	Reader 4	Reader 5	Reader 7	Reader 8	Reader 9	Reader 12	Reader 18	ALL
Stage 1a ==>	0	0.11	0.07	0.03	0.00	0.00	0.00	0.00	0.00	0.06	0.03
Stage 1b ==>	1	-0.36	-0.17	0.00	-0.21	0.00	-0.40	-0.24	-0.96	0.00	-0.26
Stage 2 ==>	2	-0.10	0.00	0.00	0.05	0.00	0.00	-0.15	-0.26	0.05	-0.05
Stage 3 ==>	3	-0.09	0.18	0.09	0.25	0.32	-0.03	0.20	0.09	0.00	0.11
Stage 4 ==>	4	-0.40	-0.14	-0.30	-0.15	-0.05	-0.45	-0.30	-0.35	-0.47	-0.29
Stage 5 ==>	5	-1.13	-0.71	-0.50	-0.43	-0.50	-0.86	-0.50	-0.71	-0.33	-0.64

Table 4.2.11. Horse mackerel eggs second staging.(A) The numbers of eggs at each modal stage read by each participant. (B) The numbers of eggs allocated to each stage by each participant. (C) The over / underestimation of stage 1 (1a+1b) by each participant. (D) The percentage agreement by modal egg stage by each participant. (E) The percentage agreement by modal stage 1a and 1b combined, by each participant. (F) The bias is indicated by the percentage over or underestimation of each egg stage, as estimated by each participant, in relation to the modal stage. For each table the combined result is also given.

HORSE MACKEREL second stageing Egg Stageing Workshop Bremerhaven, October 2018

A — NUMBER OF EGG STAGE READINGS BY MODAL EGG STAGE

	MODAL stage	Reader 1	Reader 2	Reader 3	Reader 4	Reader 5	Reader 6	Reader 7	Reader 8	Reader 9	Reader 10	Reader 11	Reader 12	Reader 13	Reader 14	Reader 15	Reader 16	Reader 17	Reader 18	Reader 19	TOTAL
Stage 1a ==>	0	27	24	26	25	28	24	8	26	24	9	26	20	30	19	25	24	25	26	25	441
Stage 1b ==>	1	-	-	-	-	-	-	-	-	-	2	-	-	-	-	-	-	-	-	-	-
Stage 2 ==>	2	5	-	9	13	8	8	15	11	12	3	4	6	4	-	12	4	1	7	2	124
Stage 3 ==>	3	13	-	18	20	20	16	17	19	17	2	6	18	7	-	20	17	2	19	4	235
Stage 4 ==>	4	2	-	3	3	3	2	2	3	2	2	-	4	2	-	3	3	-	3	2	39
Stage 5 ==>	5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Total	0-5	47	24	56	61	59	50	42	59	55	18	36	48	43	19	60	48	28	55	33	841

B — EGG STAGE COMPOSITION

	Stage	Reader 1	Reader 2	Reader 3	Reader 4	Reader 5	Reader 6	Reader 7	Reader 8	Reader 9	Reader 10	Reader 11	Reader 12	Reader 13	Reader 14	Reader 15	Reader 16	Reader 17	Reader 18	Reader 19	TOTAL
Stage 1a ==>	0	27	24	25	24	29	19	8	26	24	7	25	18	30	19	24	22	0	24	25	400
Stage 1b ==>	1	-	-	1	-	-	7	2	1	-	4	-	2	-	-	2	1	25	2	-	47
Stage 2 ==>	2	13	-	16	16	9	6	10	21	21	4	5	6	7	-	12	7	1	5	2	161
Stage 3 ==>	3	7	-	10	19	18	15	17	9	8	2	6	17	3	-	11	13	1	22	3	181
Stage 4 ==>	4	-	-	4	2	3	3	5	2	2	1	-	5	3	-	11	4	1	2	3	51
Stage 5 ==>	5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	1
Total	0-5	47	24	56	61	59	50	42	59	55	18	36	48	43	19	60	48	28	55	33	841

C — OVER- / UNDERESTIMATION OF STAGE 1 (=1A+1B)

	MODAL stage	Reader 1	Reader 2	Reader 3	Reader 4	Reader 5	Reader 6	Reader 7	Reader 8	Reader 9	Reader 10	Reader 11	Reader 12	Reader 13	Reader 14	Reader 15	Reader 16	Reader 17	Reader 18	Reader 19	ALL
	1a+1b	0%	0%	0%	-4%	4%	8%	25%	4%	0%	0%	-4%	0%	0%	0%	4%	-4%	0%	-8%	0%	1%

D — PERCENTAGE AGREEMENT BY EGG STAGE

	MODAL stage	Reader 1	Reader 2	Reader 3	Reader 4	Reader 5	Reader 6	Reader 7	Reader 8	Reader 9	Reader 10	Reader 11	Reader 12	Reader 13	Reader 14	Reader 15	Reader 16	Reader 17	Reader 18	Reader 19	ALL
Stage 1a ==>	0	96%	100%	96%	96%	100%	75%	88%	96%	96%	78%	96%	90%	100%	100%	96%	92%	0%	92%	100%	89%
Stage 1b ==>	1	-	-	-	-	-	-	-	-	-	100%	-	-	-	-	-	-	-	-	-	-
Stage 2 ==>	2	100%	-	100%	100%	88%	25%	60%	91%	100%	100%	100%	83%	100%	-	83%	100%	100%	71%	50%	83%
Stage 3 ==>	3	31%	-	56%	90%	90%	69%	82%	42%	47%	100%	100%	89%	43%	-	50%	76%	50%	100%	50%	69%
Stage 4 ==>	4	0%	-	100%	67%	100%	100%	100%	67%	100%	50%	-	100%	100%	-	67%	67%	-	67%	100%	78%
Stage 5 ==>	5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Weighted mean	0-5	74.5%	100.0%	83.9%	93.4%	94.9%	66.0%	76.2%	76.3%	81.8%	83.3%	97.2%	89.6%	90.7%	100.0%	76.7%	85.4%	7.1%	90.9%	90.9%	82.5%
	RANKING	17	1	11	5	4	18	16	15	13	12	3	9	8	1	14	10	19	6	6	

E — PERCENTAGE AGREEMENT STAGE 1A and 1B combined

	MODAL stage	Reader 1	Reader 2	Reader 3	Reader 4	Reader 5	Reader 6	Reader 7	Reader 8	Reader 9	Reader 10	Reader 11	Reader 12	Reader 13	Reader 14	Reader 15	Reader 16	Reader 17	Reader 18	Reader 19	ALL
	1a+1b	96%	100%	100%	96%	100%	96%	88%	100%	96%	100%	96%	100%	100%	100%	100%	96%	100%	100%	100%	98%
	RANKING	13	1	1	15	1	16	19	1	16	1	14	1	1	1	1	16	1	1	1	

F — BIAS

	MODAL stage	Reader 1	Reader 2	Reader 3	Reader 4	Reader 5	Reader 6	Reader 7	Reader 8	Reader 9	Reader 10	Reader 11	Reader 12	Reader 13	Reader 14	Reader 15	Reader 16	Reader 17	Reader 18	Reader 19	ALL
Stage 1a ==>	0	0.11	0.00	0.04	0.08	0.00	0.29	0.25	0.04	0.08	0.22	0.08	0.10	0.00	0.00	0.04	0.13	1.00	0.08	0.00	0.12
Stage 1b ==>	1	-	-	-	-	-	-	-	-	-	0.00	-	-	-	-	-	-	-	-	-	-
Stage 2 ==>	2	0.00	-	0.00	0.00	-0.25	0.13	-0.07	-0.18	0.00	0.00	0.00	0.17	0.00	-	0.08	0.00	0.00	0.29	0.50	0.01
Stage 3 ==>	3	-0.85	-	-0.33	-0.10	-0.10	-0.25	0.18	-0.58	-0.65	0.00	0.00	0.00	-0.29	-	0.30	0.00	0.50	0.00	0.00	-0.17
Stage 4 ==>	4	-1.00	-	0.00	-0.33	0.00	0.00	0.00	-0.33	0.00	-1.00	-	0.00	0.00	-	-0.33	0.33	-	-0.33	0.00	-0.18
Stage 5 ==>	5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Table 4.2.12. Horse mackerel eggs second staging, expert readers only.
(A) The numbers of eggs at each modal stage read by each participant. (B) The numbers of eggs allocated to each stage by each participant.
(C) The over / underestimation of stage 1 (1a+1b) by each participant. (D) The percentage agreement by modal egg stage by each participant.
(E) The percentage agreement by modal stage 1a and 1b combined, by each participant.
(F) The bias is indicated by the percentage over or underestimation of each egg stage, as estimated by each participant, in relation to the modal stage.
For each table the combined result is also given.

A

NUMBER OF EGG STAGE READINGS BY MODAL EGG STAGE

	MODAL stage	Reader 2	Reader 3	Reader 4	Reader 5	Reader 7	Reader 8	Reader 9	Reader 12	Reader 18	TOTAL
Stage 1a ==>	0	24	26	25	28	8	26	24	19	26	206
Stage 1b ==>	1	-	-	-	-	-	-	-	1	-	-
Stage 2 ==>	2	-	13	17	12	18	15	16	7	11	-
Stage 3 ==>	3	-	14	16	16	14	15	13	17	15	-
Stage 4 ==>	4	-	3	3	3	2	3	2	4	3	-
Stage 5 ==>	5	-	-	-	-	-	-	-	-	-	-
Total	0-5	24	56	61	59	42	59	55	48	55	459

B

EGG STAGE COMPOSITION

	Stage	Reader 2	Reader 3	Reader 4	Reader 5	Reader 7	Reader 8	Reader 9	Reader 12	Reader 18	TOTAL
Stage 1a ==>	0	24	25	24	29	8	26	24	18	24	202
Stage 1b ==>	1	-	1	-	-	2	1	-	2	2	8
Stage 2 ==>	2	-	16	16	9	10	21	21	6	5	104
Stage 3 ==>	3	-	10	19	18	17	9	8	17	22	120
Stage 4 ==>	4	-	4	2	3	5	2	2	5	2	25
Stage 5 ==>	5	-	-	-	-	-	-	-	-	-	-
Total	0-5	24	56	61	59	42	59	55	48	55	459

C

OVER- / UNDERESTIMATION OF STAGE 1 (=1A+1B)

MODAL stage	Reader 2	Reader 3	Reader 4	Reader 5	Reader 7	Reader 8	Reader 9	Reader 12	Reader 18	ALL
1a+1b	0%	0%	-4%	4%	25%	4%	0%	0%	-8%	2%

D

PERCENTAGE AGREEMENT BY EGG STAGE

	MODAL stage	Reader 2	Reader 3	Reader 4	Reader 5	Reader 7	Reader 8	Reader 9	Reader 12	Reader 18	ALL
Stage 1a ==>	0	100%	96%	96%	100%	88%	96%	96%	95%	92%	96%
Stage 1b ==>	1	-	-	-	-	-	-	-	100%	-	-
Stage 2 ==>	2	-	100%	88%	67%	50%	93%	100%	71%	45%	-
Stage 3 ==>	3	-	71%	100%	94%	79%	53%	62%	88%	100%	-
Stage 4 ==>	4	-	100%	67%	100%	100%	67%	100%	100%	67%	-
Stage 5 ==>	5	-	-	-	-	-	-	-	-	-	-
Weighted mean	0-5	100.0%	91.1%	93.4%	91.5%	69.0%	83.1%	89.1%	89.6%	83.6%	87.6%
RANKING		1	4	2	3	9	8	6	5	7	

E

PERCENTAGE AGREEMENT STAGE 1A and 1B combined

MODAL stage	Reader 2	Reader 3	Reader 4	Reader 5	Reader 7	Reader 8	Reader 9	Reader 12	Reader 18	ALL
1a+1b	100%	100%	96%	100%	88%	100%	96%	100%	100%	99%
RANKING	1	1	7	1	9	1	8	1	1	

F

BIAS

	MODAL stage	Reader 2	Reader 3	Reader 4	Reader 5	Reader 7	Reader 8	Reader 9	Reader 12	Reader 18	ALL
Stage 1a ==>	0	0.00	0.04	0.08	0.00	0.25	0.04	0.08	0.05	0.08	0.05
Stage 1b ==>	1	-	-	-	-	-	-	-	0.00	-	-
Stage 2 ==>	2	-	0.00	0.12	0.08	0.11	-0.13	0.00	0.29	0.55	-
Stage 3 ==>	3	-	-0.14	0.00	-0.06	0.21	-0.47	-0.54	0.00	0.00	-
Stage 4 ==>	4	-	0.00	-0.33	0.00	0.00	-0.33	0.00	0.00	-0.33	-
Stage 5 ==>	5	-	-	-	-	-	-	-	-	-	-

4.3 Results of the egg identification exercises

The same trays of eggs, which were used for egg staging, were also used for the egg identification exercises. Some of the eggs used were from artificial fertilizations and so the species of those eggs was definitely known. It was hoped that by using eggs of known species any problems associated with identification would be highlighted clearly and better descriptions of each species could be prepared.

The original assessment of species identification for each egg, by each participant, was put into a primary result table (not presented here).

Summaries of the results from the two rounds of egg species determination are presented in Tables 4.3.1 to 4.3.4. About half of the participants at the workshop were inexperienced; hence results of the expert readers are also presented separately. Each of these tables is divided into four sub-tables labelled A-D, where the performance of each participant is judged against the actual species and modal species determination.

Sub-tables A show the number of eggs at each actual or modal species that were assessed by each participant. The numbers at each modal species will therefore be the same for all participants that read all the eggs.

Sub-tables B show the numbers of eggs of each species as assessed by each participant.

Sub-tables C show the percentage under or over-estimation by each participant for each species.

Sub-tables D show the percentage agreement in species identification between the assessment of each participant and the actual or modal species.

The results highlight the difficulties in being able to positively identify eggs where there are few distinguishing features other than the size of egg and oil globule diameters, particularly in the environment of the workshop. Information that would be helpful for identification, e.g. on origin and the environmental conditions of the eggs, was not given. Also participants were not able to perform the SAT test on individual eggs in the trays. Although after the first round of analysis there was some discussion on the features which aid fish egg identification, and some references and criteria were reviewed (see section 3.3.2) to help with the identification of eggs which are similar to those of mackerel and horse mackerel, the results of the second round did not show an overall improvement of species identification (Tables 4.3.1, 4.3.2, 4.3.3 and 4.3.4). Only for mackerel eggs, the percentage agreement increased from 93% to 98% with modal/actual species and for expert readers from 97% to 99%. For horse mackerel the agreement dropped from 82% to 72% for modal/actual species and for experts from 89% to 83%.

Table 4.3.1. Species identification with modal species, first identification.
(A) The numbers of eggs at each modal stage read by each participant. (B) The numbers of eggs allocated to each stage by each participant.
(C) The over / underestimation of stage 1 (1a+1b) by each participant. (D) The percentage agreement by modal egg stage by each participant.
(E) The percentage agreement by modal stage 1a and 1b combined, by each participant.
(F) The bias is indicated by the percentage over or underestimation of each egg stage, as estimated by each participant, in relation to the modal stage. For each table the combined result is also given.

SPECIES IDENTIFICATION first determination Egg Stageing Workshop Bremerhaven, October 2018

A Species compositions using modal/actual species (second last column input table)

	Modal or actual species	Reader 1	Reader 2	Reader 3	Reader 4	Reader 5	Reader 6	Reader 7	Reader 8	Reader 9	Reader 10	Reader 11	Reader 12	Reader 13	Reader 14	Reader 15	Reader 16	Reader 17	Reader 18	Reader 19	Reader 20	Reader 21	TOTAL
Mackerel	1	206	205	206	206	205	206	206	206	207	206	197	207	206	206	206	205	207	205	206	206	205	4315
Horse Mackerel	2	35	35	35	35	35	35	35	35	35	35	35	35	35	35	35	35	35	35	35	35	32	732
Megrim	3	11	11	11	12	12	11	11	11	12	12	12	12	12	12	12	11	11	12	10	12	11	241
Hake	4	21	21	21	21	19	21	21	21	21	21	19	20	21	21	21	21	21	21	18	21	20	432
Other species	5	23	23	24	23	24	23	24	24	23	23	20	23	24	24	24	23	23	24	22	24	22	487
Total	1-5	296	295	297	297	295	296	297	297	298	297	283	297	298	298	298	295	297	297	291	298	290	6207

B Species compositions as estimated per participant and whole group

	Species	Reader 1	Reader 2	Reader 3	Reader 4	Reader 5	Reader 6	Reader 7	Reader 8	Reader 9	Reader 10	Reader 11	Reader 12	Reader 13	Reader 14	Reader 15	Reader 16	Reader 17	Reader 18	Reader 19	Reader 20	Reader 21	TOTAL
Mackerel	1	207	136	218	214	205	206	202	220	202	219	196	199	203	208	216	216	171	204	240	199	166	4247
Horse Mackerel	2	36	82	34	33	34	37	45	27	50	29	45	58	45	43	37	36	81	41	34	36	65	928
Megrim	3	-	-	-	-	8	-	2	10	26	7	2	-	-	2	3	-	11	1	-	15	-	87
Hake	4	24	12	25	25	26	22	17	21	3	19	14	13	10	27	-	1	19	12	-	19	10	319
Other species	5	29	65	20	25	22	31	31	19	17	23	26	27	40	18	42	42	15	39	17	29	49	626
Total	1-5	296	295	297	297	295	296	297	297	298	297	283	297	298	298	298	295	297	297	291	298	290	6207

C Percentage overestimation / underestimation

	Modal or actual species	Reader 1	Reader 2	Reader 3	Reader 4	Reader 5	Reader 6	Reader 7	Reader 8	Reader 9	Reader 10	Reader 11	Reader 12	Reader 13	Reader 14	Reader 15	Reader 16	Reader 17	Reader 18	Reader 19	Reader 20	Reader 21	ALL
Mackerel	1	0%	-34%	6%	4%	0%	0%	-2%	7%	-2%	6%	-1%	-4%	-1%	1%	5%	5%	-17%	0%	17%	-3%	-19%	-2%
Horse Mackerel	2	3%	134%	-3%	-6%	-3%	6%	29%	-23%	43%	-17%	29%	66%	29%	23%	6%	3%	131%	17%	-3%	3%	103%	27%
Megrim	3	-	-	-	-	-	-33%	-	-82%	-9%	117%	-42%	-83%	-	-	-83%	-75%	-	0%	-92%	-	-25%	-	-64%
Hake	4	14%	-43%	19%	19%	37%	5%	-19%	0%	-86%	-10%	-26%	-35%	-52%	29%	#WERT!	-95%	-10%	-43%	-	-10%	-50%	-26%
Other species	5	26%	183%	-17%	9%	-8%	35%	29%	-21%	-26%	0%	30%	17%	67%	-25%	75%	83%	-35%	63%	-23%	21%	123%	29%

D Percentage agreement in species identification per species

	Modal or actual species	Reader 1	Reader 2	Reader 3	Reader 4	Reader 5	Reader 6	Reader 7	Reader 8	Reader 9	Reader 10	Reader 11	Reader 12	Reader 13	Reader 14	Reader 15	Reader 16	Reader 17	Reader 18	Reader 19	Reader 20	Reader 21	ALL
Mackerel	1	99%	64%	100%	99%	99%	99%	94%	100%	89%	100%	93%	91%	96%	92%	96%	100%	75%	98%	100%	95%	77%	93%
Horse Mackerel	2	94%	91%	94%	91%	94%	94%	80%	66%	26%	80%	91%	71%	94%	91%	83%	94%	80%	94%	94%	91%	28%	82%
Megrim	3	0%	0%	0%	0%	50%	0%	0%	0%	8%	50%	17%	0%	0%	0%	0%	0%	18%	0%	0%	100%	0%	12%
Hake	4	57%	19%	76%	67%	84%	71%	43%	76%	0%	81%	53%	35%	29%	48%	0%	5%	71%	48%	0%	71%	5%	45%
Other species	5	96%	52%	79%	83%	79%	91%	54%	79%	74%	91%	95%	78%	92%	71%	71%	91%	39%	79%	77%	75%	77%	77%
Weighted mean	1-5	91.2%	61.0%	91.9%	90.2%	93.9%	91.9%	82.2%	88.9%	71.1%	93.6%	87.3%	80.1%	86.9%	83.2%	81.9%	88.1%	70.7%	88.2%	88.0%	91.6%	63.4%	84.1%
	RANKING	6	21	3	7	1	4	15	8	18	2	12	17	13	14	16	10	19	9	11	5	20	

Table 4.3.2. Species identification with modal species, first identification, expert readers only.
(A) The numbers of eggs at each modal stage read by each participant. (B) The numbers of eggs allocated to each stage by each participant.
(C) The over / underestimation of stage 1 (1a+1b) by each participant. (D) The percentage agreement by modal egg stage by each participant.
(E) The percentage agreement by modal stage 1a and 1b combined, by each participant.
(F) The bias is indicated by the percentage over or underestimation of each egg stage, as estimated by each participant, in relation to the modal stage. For each table the combined result is also given.

A Species compositions using modal/actual species

	Modal or actual species	Reader 1	Reader 3	Reader 4	Reader 5	Reader 6	Reader 8	Reader 10	Reader 11	Reader 14	Reader 20	TOTAL
Mackerel	1	206	206	206	205	206	206	206	197	206	206	**2050**
Horse Mackerel	2	35	35	35	35	35	35	35	35	35	35	**350**
Megrim	3	11	11	12	12	11	11	12	12	12	12	**116**
Hake	4	21	21	21	19	21	21	21	19	21	21	**206**
Other species	5	23	24	23	24	23	24	23	20	24	24	**232**
Total	1-5	**296**	**297**	**297**	**295**	**296**	**297**	**297**	**283**	**298**	**298**	**2954**

B Species compositions as estimated per participant and whole group

	Species	Reader 1	Reader 3	Reader 4	Reader 5	Reader 6	Reader 8	Reader 10	Reader 11	Reader 14	Reader 20	TOTAL
Mackerel	1	207	218	214	205	206	220	219	196	208	199	**2092**
Horse Mackerel	2	36	34	33	34	37	27	29	45	43	36	**354**
Megrim	3	-	-	-	8	-	10	7	2	2	15	**44**
Hake	4	24	25	25	26	22	21	19	14	27	19	**222**
Other species	5	29	20	25	22	31	19	23	26	18	29	**242**
Total	1-5	**296**	**297**	**297**	**295**	**296**	**297**	**297**	**283**	**298**	**298**	**2954**

C Percentage overestimation / underestimation

	Modal or actual species	Reader 1	Reader 3	Reader 4	Reader 5	Reader 6	Reader 8	Reader 10	Reader 11	Reader 14	Reader 20	ALL
Mackerel	1	0%	6%	4%	0%	0%	7%	6%	-1%	1%	-3%	**2%**
Horse Mackerel	2	3%	-3%	-6%	-3%	6%	-23%	-17%	29%	23%	3%	**1%**
Megrim	3	-	-	-	-33%	-	-9%	-42%	-83%	-83%	25%	**-62%**
Hake	4	14%	19%	19%	37%	5%	0%	-10%	-26%	29%	-10%	**8%**
Other species	5	26%	-17%	9%	-8%	35%	-21%	0%	30%	-25%	21%	**4%**

D Percentage agreement in species identification per species

	Modal or actual species	Reader 1	Reader 3	Reader 4	Reader 5	Reader 6	Reader 8	Reader 10	Reader 11	Reader 14	Reader 20	ALL
Mackerel	1	99%	100%	99%	99%	99%	100%	100%	93%	92%	95%	**97%**
Horse Mackerel	2	94%	94%	91%	94%	94%	66%	80%	91%	91%	91%	**89%**
Megrim	3	0%	0%	0%	50%	0%	0%	50%	17%	0%	100%	**22%**
Hake	4	57%	76%	67%	84%	71%	76%	81%	53%	48%	71%	**68%**
Other species	5	96%	79%	83%	79%	91%	79%	91%	95%	71%	75%	**84%**
Weighted mean	1-5	**91.2%**	**91.9%**	**90.2%**	**93.9%**	**91.9%**	**88.9%**	**93.6%**	**87.3%**	**83.2%**	**91.6%**	**90.4%**
	RANKING	**6**	**3**	**7**	**1**	**4**	**8**	**2**	**9**	**10**	**5**	

Table 4.3.3. Species identification with modal species, second identification.
(A) The numbers of eggs at each modal stage read by each participant. (B) The numbers of eggs allocated to each stage by each participant.
(C) The over / underestimation of stage 1 (1a+1b) by each participant. (D) The percentage agreement by modal egg stage by each participant.
(E) The percentage agreement by modal stage 1a and 1b combined, by each participant.
(F) The bias is indicated by the percentage over or underestimation of each egg stage, as estimated by each participant, in relation to the modal stage. For each table the combined result is also given.

SPECIES IDENTIFICATION second determination Egg Stageing Workshop Bremerhaven, October 2018

A **Species compositions using modal/actual species (second last column input table)**

	Modal or actual species	Reader 1	Reader 2	Reader 3	Reader 4	Reader 5	Reader 6	Reader 7	Reader 8	Reader 9	Reader 10	Reader 11	Reader 12	Reader 13	Reader 14	Reader 15	Reader 16	Reader 17	Reader 18	Reader 19	TOTAL
Mackerel	1	132	132	131	132	132	132	132	131	132	132	132	132	132	132	132	132	131	132	131	**2504**
Horse Mackerel	2	54	54	54	54	54	54	54	54	53	54	54	54	54	54	54	54	54	54	54	**1025**
Megrim	3	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	9	10	10	**189**
Hake	4	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15	**285**
Other species	5	26	24	27	27	27	27	27	26	27	26	27	27	27	27	27	27	27	27	27	**507**
Total	1-5	237	235	237	238	238	238	238	236	237	237	238	238	238	238	238	238	236	238	237	**4510**

B **Species compositions as estimated per participant and whole group**

	Species	Reader 1	Reader 2	Reader 3	Reader 4	Reader 5	Reader 6	Reader 7	Reader 8	Reader 9	Reader 10	Reader 11	Reader 12	Reader 13	Reader 14	Reader 15	Reader 16	Reader 17	Reader 18	Reader 19	TOTAL
Mackerel	1	154	145	153	146	137	142	144	141	146	149	136	157	141	148	137	143	143	134	155	**2751**
Horse Mackerel	2	47	24	56	61	59	50	42	59	55	18	36	48	43	19	60	48	28	55	33	**841**
Megrim	3	5	1	4	-	9	2	30	-	-	36	4	4	3	4	11	10	29	10	5	**167**
Hake	4	8	51	6	6	15	26	4	10	6	23	-	17	33	46	8	8	10	15	17	**309**
Other species	5	23	14	18	25	18	18	18	26	30	11	62	12	18	21	22	29	26	24	27	**442**
Total	1-5	237	235	237	238	238	238	238	236	237	237	238	238	238	238	238	238	236	238	237	**4510**

C **Percentage overestimation / underestimation**

	Modal or actual species	Reader 1	Reader 2	Reader 3	Reader 4	Reader 5	Reader 6	Reader 7	Reader 8	Reader 9	Reader 10	Reader 11	Reader 12	Reader 13	Reader 14	Reader 15	Reader 16	Reader 17	Reader 18	Reader 19	ALL
Mackerel	1	17%	10%	17%	11%	4%	8%	9%	8%	11%	13%	3%	19%	7%	12%	4%	8%	9%	2%	18%	**10%**
Horse Mackerel	2	-13%	-56%	4%	13%	9%	-7%	-22%	9%	4%	-67%	-33%	-11%	-20%	-65%	11%	-11%	-48%	2%	-39%	**-18%**
Megrim	3	-50%	-90%	-60%	-	-10%	-80%	200%	-	-	260%	-60%	-60%	-70%	-60%	10%	0%	222%	0%	-50%	**-12%**
Hake	4	-47%	240%	-60%	-60%	0%	73%	-73%	-33%	-60%	53%	-	13%	120%	207%	-47%	-47%	-33%	0%	13%	**8%**
Other species	5	-12%	-42%	-33%	-7%	-33%	-33%	-33%	0%	11%	-58%	130%	-56%	-33%	-22%	-19%	7%	-4%	-11%	0%	**-13%**

D **Percentage agreement in species identification per species**

	Modal or actual species	Reader 1	Reader 2	Reader 3	Reader 4	Reader 5	Reader 6	Reader 7	Reader 8	Reader 9	Reader 10	Reader 11	Reader 12	Reader 13	Reader 14	Reader 15	Reader 16	Reader 17	Reader 18	Reader 19	ALL
Mackerel	1	100%	100%	98%	100%	98%	98%	99%	100%	100%	98%	98%	99%	95%	100%	94%	100%	99%	95%	99%	**98%**
Horse Mackerel	2	76%	44%	94%	98%	100%	87%	52%	98%	91%	6%	65%	69%	67%	33%	96%	87%	52%	98%	57%	**72%**
Megrim	3	30%	0%	10%	0%	90%	0%	80%	0%	0%	0%	30%	0%	0%	30%	90%	90%	89%	90%	30%	**34%**
Hake	4	27%	93%	33%	40%	67%	47%	0%	60%	33%	40%	0%	27%	87%	93%	20%	33%	33%	80%	33%	**45%**
Other species	5	35%	46%	52%	56%	63%	52%	56%	65%	59%	38%	89%	44%	44%	56%	67%	67%	56%	67%	67%	**57%**
Weighted mean	1-5	**79.7%**	**77.0%**	**84.0%**	**86.6%**	**92.0%**	**82.8%**	**76.5%**	**89.0%**	**84.8%**	**62.4%**	**80.7%**	**77.3%**	**78.6%**	**76.5%**	**86.6%**	**88.7%**	**78.8%**	**91.2%**	**78.9%**	**81.7%**
	RANKING	**11**	**16**	**8**	**5**	**1**	**9**	**17**	**3**	**7**	**19**	**10**	**15**	**14**	**17**	**5**	**4**	**13**	**2**	**12**	

Table 4.3.4. Species identification with modal species, second identification, expert readers only.
(A) The numbers of eggs at each modal stage read by each participant. (B) The numbers of eggs allocated to each stage by each participant.
(C) The over / underestimation of stage 1 (1a+1b) by each participant. (D) The percentage agreement by modal egg stage by each participant.
(E) The percentage agreement by modal stage 1a and 1b combined, by each participant.
(F) The bias is indicated by the percentage over or underestimation of each egg stage, as estimated by each participant, in relation to the modal stage. For each table the combined result is also given.

A **Species compositions using modal/actual species**

	Modal or actual species	Reader 2	Reader 3	Reader 4	Reader 5	Reader 7	Reader 8	Reader 9	Reader 12	Reader 18	TOTAL
Mackerel	1	132	131	132	132	132	131	132	132	132	1186
Horse Mackerel	2	54	54	54	54	54	54	53	54	54	485
Megrim	3	10	10	10	10	10	10	10	10	10	90
Hake	4	15	15	15	15	15	15	15	15	15	135
Other species	5	24	27	27	27	27	26	27	27	27	239
Total	1-5	235	237	238	238	238	236	237	238	238	2135

B **Species compositions as estimated per participant and whole group**

	Species	Reader 2	Reader 3	Reader 4	Reader 5	Reader 7	Reader 8	Reader 9	Reader 12	Reader 18	TOTAL
Mackerel	1	145	153	146	137	144	141	146	157	134	1303
Horse Mackerel	2	24	56	61	59	42	59	55	48	55	459
Megrim	3	1	4	-	9	30	-	-	4	10	58
Hake	4	51	6	6	15	4	10	6	17	15	130
Other species	5	14	18	25	18	18	26	30	12	24	185
Total	1-5	235	237	238	238	238	236	237	238	238	2135

C **Percentage overestimation / underestimation**

	Modal or actual species	Reader 2	Reader 3	Reader 4	Reader 5	Reader 7	Reader 8	Reader 9	Reader 12	Reader 18	ALL
Mackerel	1	10%	17%	11%	4%	9%	8%	11%	19%	2%	10%
Horse Mackerel	2	-56%	4%	13%	9%	-22%	9%	4%	-11%	2%	-5%
Megrim	3	-90%	-60%	-	-10%	200%	-	-	-60%	0%	-36%
Hake	4	240%	-60%	-60%	0%	-73%	-33%	-60%	13%	0%	-4%
Other species	5	-42%	-33%	-7%	-33%	-33%	0%	11%	-56%	-11%	-23%

D **Percentage agreement in species identification per species**

	Modal or actual species	Reader 2	Reader 3	Reader 4	Reader 5	Reader 7	Reader 8	Reader 9	Reader 12	Reader 18	ALL
Mackerel	1	100%	98%	100%	98%	99%	100%	100%	99%	95%	99%
Horse Mackerel	2	44%	94%	98%	100%	52%	98%	91%	69%	98%	83%
Megrim	3	0%	10%	0%	90%	80%	0%	0%	0%	90%	30%
Hake	4	93%	33%	40%	67%	0%	60%	33%	27%	80%	48%
Other species	5	46%	52%	56%	63%	56%	65%	59%	44%	67%	56%
Weighted mean	1-5	77.0%	84.0%	86.6%	92.0%	76.5%	89.0%	84.8%	77.3%	91.2%	84.3%
	RANKING	8	6	4	1	9	3	5	7	2	

4.4 Results of the fecundity, atresia and POF staging exercises (ToR e)

During the fixative exercise, it was concluded that fixation with formaline was better than with Finefix. Staging of oocytes was more complicated in Finefix compared with formaline. POFs and atresia analyses might be difficult in Finefix.

In general, results of fresh material were better compared to frozen gonads both with formaline and Finefix. However, Ultrafrozen formaline fixed samples provided good quality histology, which is an important result when processing samples from the fishery. The freezing process damages the cells in the ovary samples, but with the Ultrafreezing proces the fish, with the gonads separately, are rapidly frozen. The Ultrafrozen samples were from the freezer immediately put in formaldehyde, without thawing. The fast freezing process and non-thawing enhanced the quality of the histological samples considerably. These histological samples allowed for staging of oocytes.

4.4.1 Results of the ovary screening and analysis

The discussed Hydration_stage and Egg_stage new options to choose from were added into the screening template. For Hydration_stage now four options are displayed: 0_no hydrated oocytes present, 1_early hydration, 2_advanced hydration and 3_late hydration. This used to be only 0_no hydrated oocytes present and 1_hydrated oocytes present. For Egg_stage also four new stages were introduced: 0_eggs not present, 1_new eggs present, 2_residual eggs present and 3_new and residual eggs present. These stages are essential to better distinguish the females which are actually spawning.

15 readers examined 15 screening slides. The average overall agreement for Hydration_stage was 90 %, for Early_alpha atresia was 89 %, for massive atresia was 92 %, for spent eggs was 90 %, for egg_stage was 80 % and for POF was 75 % (Table 4.4.1.1). As not all the participants are experienced the results for first 4 criteria look promising, the average overall agreement is around 90 %. For Egg_stage and POF the average overall agreement was lower and needs to be improved. For Egg_stage it seemed that the new system with 4 options and the criteria to distinguish them were not clear enough for all participants (Figure 4.4.1.1). Participants also had problems to recognize presence/absence of POFs in the slides, which is supposed to be easier than staging of POFs. Most likely the exercise of the POF staging will be beneficial for participants to recognize POFs and improve this skill for the actual survey next year.

There was disagreement in Spent in some samples, basically due to the different interpretation of the phase of the reproductive cycle at which the ovary was captured, i.e. recovery and again developing new oocytes versus spent and some oocytes left that will later enter atresia. It was decided that even if cortical alveoli oocytes are present in ovaries apparently at Spent, they are classified at Spent.

Table 4.4.1.1. Results of the screening analyses exercise.

type	Sample_ref	mode	frecuency	Perc_Agreement
Hydration_state_H	S01	0	14	100
Hydration_state_H	S02	0	11	73.3
Hydration_state_H	S03	1	13	86.7
Hydration_state_H	S04	0	14	100
Hydration_state_H	S05	0	14	93.3
Hydration_state_H	S06	0	15	100
Hydration_state_H	S07	0	15	100
Hydration_state_H	S08	0	13	100
Hydration_state_H	S09	0	13	100
Hydration_state_H	S10	0	13	100
Hydration_state_H	S11	0	15	100
Hydration_state_H	S12	2	10	66.7
Hydration_state_H	S13	2	9	60
Hydration_state_H	S14	3	12	80
Hydration_state_H	S15	3	14	93.3

type	Sample_ref	mode	frecuency	Perc_Agreement
Egg_stage_H	S01	0	14	100
Egg_stage_H	S02	1	11	73.3
Egg_stage_H	S03	0	15	100
Egg_stage_H	S04	0	14	100
Egg_stage_H	S05	1	7	46.7
Egg_stage_H	S06	0	15	100
Egg_stage_H	S07	0	15	100
Egg_stage_H	S08	0	13	100
Egg_stage_H	S09	0	11	84.6
Egg_stage_H	S10	0	13	100
Egg_stage_H	S11	0	15	100
Egg_stage_H	S12	0	8	53.3
Egg_stage_H	S13	0	8	53.3
Egg_stage_H	S14	0	7	46.7
Egg_stage_H	S15	1	7	46.7

type	Sample_ref	mode	frecuency	Perc_Agreement
POF_H	S01	0	12	85.7
POF_H	S02	0	11	73.3
POF_H	S03	0	13	86.7
POF_H	S04	0	8	57.1
POF_H	S05	1	10	66.7
POF_H	S06	0	9	60
POF_H	S07	0	11	73.3
POF_H	S08	0	9	69.2
POF_H	S09	0	12	92.3
POF_H	S10	1	7	53.8
POF_H	S11	0	14	93.3
POF_H	S12	1	10	66.7
POF_H	S13	1	12	80
POF_H	S14	1	13	86.7
POF_H	S15	1	14	93.3

type	Sample_ref	mode	frecuency	Perc_Agreement
Massive_atr_H	S01	1	9	64.3
Massive_atr_H	S02	0	15	100
Massive_atr_H	S03	0	14	93.3
Massive_atr_H	S04	0	14	100
Massive_atr_H	S05	0	15	100
Massive_atr_H	S06	0	14	93.3
Massive_atr_H	S07	0	8	53.3
Massive_atr_H	S08	0	13	100
Massive_atr_H	S09	0	13	100
Massive_atr_H	S10	0	10	76.9
Massive_atr_H	S11	0	15	100
Massive_atr_H	S12	0	15	100
Massive_atr_H	S13	0	15	100
Massive_atr_H	S14	0	15	100
Massive_atr_H	S15	0	15	100

type	Sample_ref	mode	frecuency	Perc_Agreement
Early_alpha_H	S01	0	14	100
Early_alpha_H	S02	1	12	80
Early_alpha_H	S03	1	13	86.7
Early_alpha_H	S04	0	12	85.7
Early_alpha_H	S05	0	14	93.3
Early_alpha_H	S06	0	15	100
Early_alpha_H	S07	1	10	66.7
Early_alpha_H	S08	0	13	100
Early_alpha_H	S09	1	9	69.2
Early_alpha_H	S10	0	13	100
Early_alpha_H	S11	0	15	100
Early_alpha_H	S12	1	10	66.7
Early_alpha_H	S13	0	15	100
Early_alpha_H	S14	0	15	100
Early_alpha_H	S15	0	15	100

type	Sample_ref	mode	frecuency	Perc_Agreement
Spent_H	S01	1	14	100
Spent_H	S02	0	15	100
Spent_H	S03	0	15	100
Spent_H	S04	0	13	92.9
Spent_H	S05	0	14	93.3
Spent_H	S06	0	15	100
Spent_H	S07	0	10	66.7
Spent_H	S08	0	12	92.3
Spent_H	S09	0	13	100
Spent_H	S10	1	7	53.8
Spent_H	S11	0	9	60
Spent_H	S12	0	15	100
Spent_H	S13	0	15	100
Spent_H	S14	0	15	100
Spent_H	S15	0	15	100

Based on the results of the screening exercise, few samples with the highest disagreement in several stages were discussed in plenary (Figure 4.4.1.1):

- S04: Half of the readers noted presence of POF and half not. It was agreed that these structures classified as POFs were old stages of POFs in any case, which do not affect the sample selection for the spawning fraction estimation.
- S10: There was no massive atresia, due to that almost all vitellogenic were reabsorbed.
- S14: There was no Egg_stage but Hydration_stage because almost all hydrated oocytes were surrounded by follicle layer and no recent postovulatory follicles were present.
- S15: There was no Egg_stage but Hydration_stage because almost all hydrated oocytes were surrounded by follicle layer and no recent postovulatory follicles were present.

Description for a few stages are clarified from those included in the manual (SISP 5) in order to improve the identification of them:

Spent: a fish is spent when it will not spawn any more in the present reproductive season. Fish are sexually mature but reproductively inactive. Typically, ovaries are characterised by atresia, abundant capillaries with blood cells, possibly POFs, and few

(if any) healthy vitellogenic oocytes; in a more advanced spent phase, the most advanced oocyte stage is PG oocyte (potentially CA), late-stage atresia, and a thicker ovarian membrane than is seen in immature fish.

Egg: A hyaline egg is fully ovulated, and the follicle layer is not around the cell anymore. Besides, recent POFs are present too. Otherwise it is considered a hydrated oocyte or a residual egg.

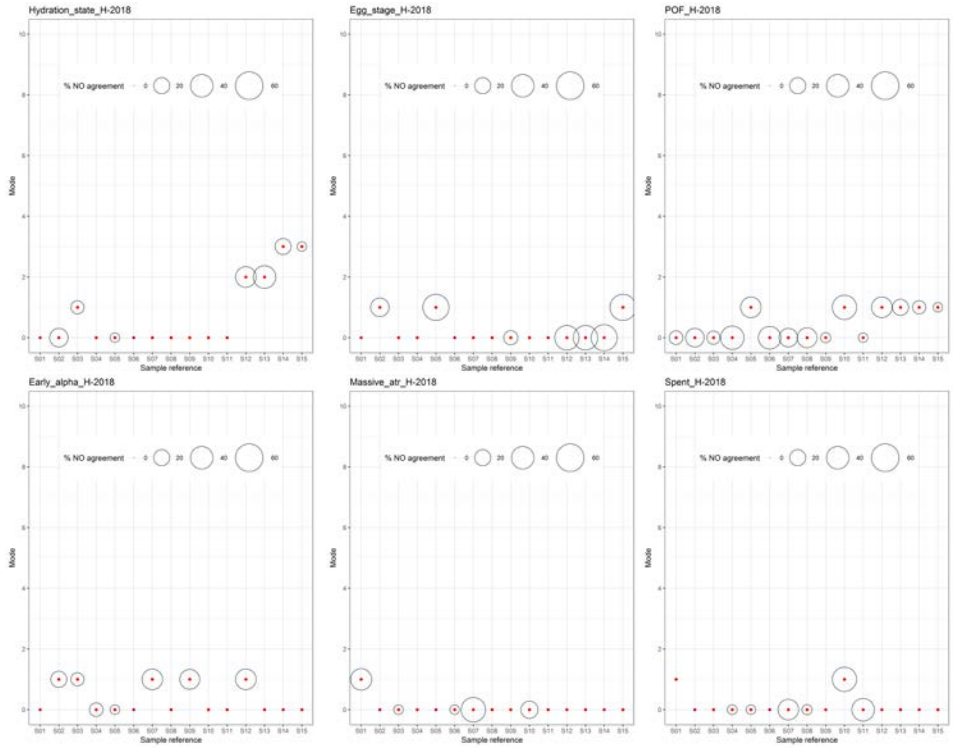


Figure 4.4.1.1. Mode and percentage of non-agreement among readers for each of the markers of screening; where red point is the mode and the size of the black bubble is the percentage of non- agreement.

The results obtained within a comparative study between spoon method and section method showed that both method are similar regarding the detection of Migratory nucleus stage and POFs, but not residual eggs; the spoon method target this stage on fewer occasions. Besides, there are significant differences between both methods when POF staging; stages tend to be earlier in the spoon method than in the section method. This is probably due to tissue arrangement into the histoblocks. It is necessary to remember that the classification criterion is based on the section method.

4.4.2 The Results of the POF staging criteria and analysis

11 readers analysed 11 slides of about 17. 6 slides were excluded do it tight schedule. The overall agreement for the slides was only 47 % (Table 4.4.2.1). To identify POFs 7 stages are being used although for the analyses they are later decreased to only 3 daily stages. None of the slides had an overall agreement of 100% and the sample P11 had only an agreement of 18 % (Figure 4.4.2.1). Not only is it difficult to see the differences in POF stages but also to distinguish POFs from atresia.

Table 4.4.2.1. Results of the screening analyses exercise.

type	Sample_ref	mode	frecuency	Perc_Agreement
POF.Stage	P10	3	5	45.5
POF.Stage	P11	3	2	18.2
POF.Stage	P12	5	4	36.4
POF.Stage	P13	2	7	63.6
POF.Stage	P14	0	4	36.4
POF.Stage	P15	3	8	72.7
POF.Stage	P16	3	6	60
POF.Stage	P17	1	5	50
POF.Stage	P7	3	5	45.5
POF.Stage	P8	3	2	66.7
POF.Stage	P9	4	5	45.5
POF.Stage	P10	3	5	45.5
POF.Stage	P11	3	2	18.2
POF.Stage	P12	5	4	36.4
POF.Stage	P13	2	7	63.6

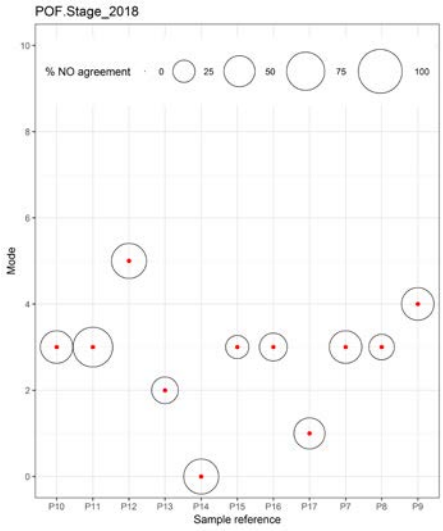


Figure 4.4.2.1. Mode and percentage of non-agreement among readers for POF stage by sample; where red point is the mode and the size of the black bubble is the variance.

Due to the high disagreement (Figure 4.4.2.2), three samples were revised in plenary:

- P11: This sample showed the highest variance among participant.
- P13: This sample showed a clear mode although still different stages were identified above and below the mode. POF stage was not further than day 1: the lumen is clearly visible, and theca and granulosa are still quite well arranged.
- P14: This sample was divided between readers staged as POF being present or absent. Late POFs may be confused with atresia, but there is no problem between no POF and very late ones in relation either to fecundity calculations or Spawning fraction estimation.

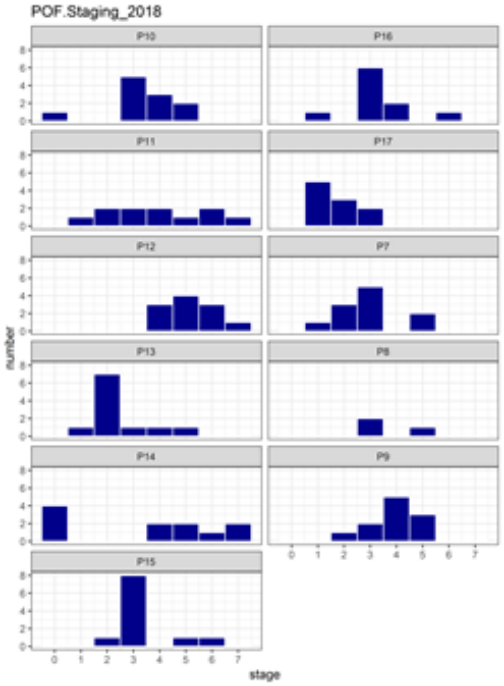


Figure 4.4.2.2. Frequency distribution of POF stages classified by readers in the samples.

4.4.3 The Results of alpha atresia image analysis

1 ovary sample was used in the calibration exercise. 11 readers examined the sample corresponding to 3 images of histological slide. The number of points counted by participants ranged from 58 to 115, while the number of profiles did from 6 to 17 (Table 4.4.3.1). The number of points out of the ovary section, i.e. negative grid, ranged from 15 to 45.

Table 4.4.3.1. Results of the atresia exercise.

Reader	YV	YV-YG	YG	NegGrid	Extra	YV-P	YV-YG-P	YG-P
reader_01	31	35	33	30	0	4	4	5
reader_02	0	0	78	43	0	0	0	0
reader_03	0	94	0	31	0	0	11	0
reader_04	0	58	0	32	0	0	6	0
reader_06	17	68	0	27	0	3	9	0
reader_05	0	95	20	44	0	0	9	1
reader_07	0	0	92	38	0	0	0	11
reader_08	0	75	0	43	0	0	6	11
reader_09	14	59	0	14	0	2	7	0
reader_10	66	45	0	45	0	8	6	0
Plenary	0	93	0	44	0	0	9	0

Due to high variance (Figure 4.4.3.1), image by image was discussed and agreed both points and profile counting in plenary. The total number of points was 93 and number of profiles 9 (Table 4.4.3.1 and "@" in Figure 4.4.3.1. Violin plot for the scores in both the number of points and the number of profiles, where "@" is the score in the joint exercise (Violin plots allow to visualize the distribution of a numeric variable using the kernel probability density of the data at different values). Right plots show the range of the scores (red bars) as well as the mean (blue segment) and the standard deviation (empty boxes) for both number of points and number of profiles.). Negative grid was agreed to be 44.

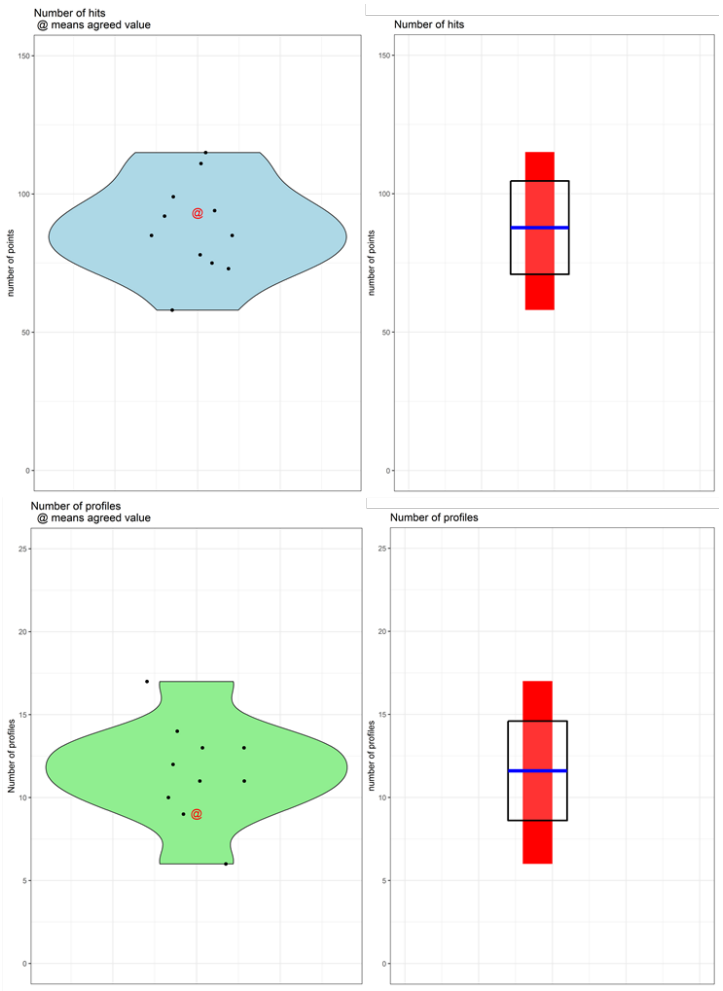


Figure 4.4.3.1. Violin plot for the scores in both the number of points and the number of profiles, where "@" is the score in the joint exercise (Violin plots allow to visualize the distribution of a numeric variable using the kernel probability density of the data at different values). Right plots show the range of the scores (red bars) as well as the mean (blue segment) and the standard deviation (empty boxes) for both number of points and number of profiles.

4.4.4 Results of potential fecundity image analysis

11 readers analysed 1 whole mount sample corresponding to 4 pictures. Automatic counting turned out quite well where the number of oocytes ranged from 524 to 532 with same mean diameter (Table 4.4.4.1 and Figure 4.4.4.1). However, manual counting of number of oocytes showed a wider range between 126 and 236 (Table 4.4.4.1 and Figure 4.4.4.2).

Table 4.4.4.1. Results of the fecundity exercise.

Manual counts							Automatic counts					
reader	mode	max_diam	min_diam	mean_diam	number		reader	mode	max_diam	min_diam	mean_diam	number
reader_11	185	185	185	185	158		reader_11	440	548	250	402	531
reader_01	185	185	185	185	185		reader_01	440	548	250	402	524
reader_02	185	185	185	185	128		reader_02	440	548	250	402	531
reader_03	185	185	185	185	185		reader_03	440	548	250	402	532
reader_04	185	185	185	185	147		reader_04	440	548	250	402	530
reader_06	185	185	185	185	126		reader_06	440	548	250	402	529
reader_05	185	185	185	185	158		reader_05	440	548	250	402	532
reader_07	185	185	185	185	236		reader_07	440	548	250	402	531
reader_08	185	185	185	185	208		reader_08	440	548	250	402	528
reader_09	185	185	32	184	146		reader_09	440	548	250	402	531
reader_10	185	185	185	185	205		reader_10	440	548	250	402	530

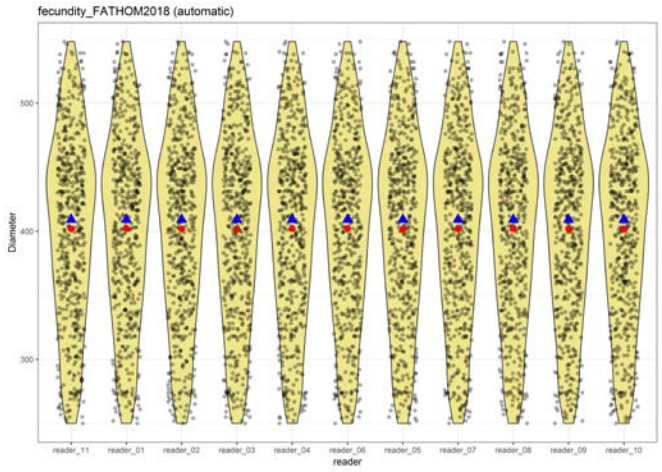


Figure 4.4.4.1. Violin plot for automatic counting results, where the red dot is the mean diameter and the blue triangle is the mode. Violin plots allow to visualize the distribution of a numeric variable using the kernel probability density of the data at different values.

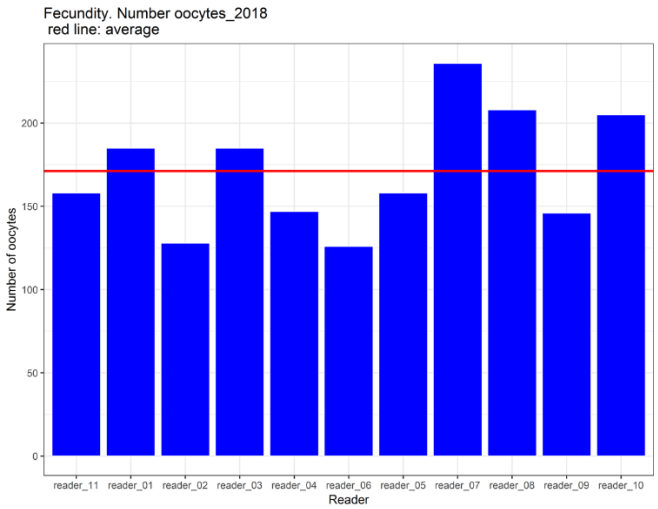


Figure 4.4.4.2. Number of ovocites estimated in the whole mount sample using manual counting method; where the red line is the average number of oocytes among readers.

It was concluded that when manually counting, there is high variance on the number of oocytes due to probable differences on identifying the oocytes above 185 micron among readers.

4.4.5 Results of micropipette sampling

The micropipette exercise was particularly useful for those WK participants who in the past had problems collecting the ovary pipette samples for fecundity as well as for those, who will undertake that sampling for the first time in 2019. It appeared that the problems with the pipette sampling in the past occured due to malfunctioning plungers. New plungers were available at the WK and all participants were able to experience the difference between a malfunctioning and correctly functioning pipette plungers.

4.5 Update the set of standard pictures for both oocytes and POFs stages (ToR f)

The description and accompanying pictures for both oocyte development stages and POFs stages have been updated during the workshop and included accordingly in the fecundity manual SISP 5.

4.6 Harmonize the analysis and interpretation of fecundity and atresia analysis (ToR g)

As described above, during the workshop the analysis and interpretation not only of fecundity and atresia but also the previous screening analyses were harmonized. POFs staging remained difficult to harmonize but the updates of the manual w.r.t. the description as well as the pictures will hopefully improve the analysis.

4.7 Review the methodology in use and available documentation on fecundity determination in order to redefine standard protocols (ToR h)

All the material, documentation and protocol, were reviewed during the workshop and new methodology was introduced i.e. NPD.viewer2, to the satisfaction of the participants because it improved comfort of the working and the quality of the results.

5 Discussion

In a plenary session it was discussed what the results of the workshop represent and if results could be used in the assessment of the total egg productions. The goal of WKFATHOM is to refresh the analysts participating in the mackerel and horse mackerel egg surveys. The surveys are carried out triennially and for most survey participants egg identification and staging and fecundity estimation are only carried out in the survey year. Hence it is necessary for survey participants to prepare before going on the survey. Therefore, the results of these workshops should not be used as an indication of the actual egg identification and staging and fecundity estimation. To achieve just this, ring tests should be carried out during or after the survey to assess the performance of survey participants.

For new participants to the survey, the WKFATHOM workshops can be a first acquaintance with egg identification and staging as well as fecundity analyses. However, it should be realized that one week of egg staging and identification and/or one week of fecundity and atresia estimation is not a full course to create experts in these fields. It is the responsibility of the individual participating institutes that (new) survey participants receive the required training.

5.1 Egg sorting exercise and SAT test

During the egg workshop two spray exercises were done with samples that contained as well mackerel as hake eggs. The sample was sprayed three times and the remaining part of the sample was also analysed for the presence of eggs. If eggs were found a SAT test was done.

The results of the first round showed that the participants managed to find at least 96% of the eggs that were in the sample. The SAT test showed, however, that not only hake eggs were floating but some mackerel eggs as well. Besides this, still a high percentage of eggs were found in the remainder of the sample after spraying. The eggs that were used in this experiment were fairly old so it was decided to use "fresher" eggs for the second round.

In the second round the percentage of eggs found by some participants decreased, the lowest percentage of recovered eggs was 85%. The results of the SAT tests were more according to what was expected. Most of the eggs that were floating in the SAT test were found in the remainder of the sample after the spraying and were probably hake eggs. Under the microscope, it also became apparent, that while applying the SAT, hake eggs emerged partly through the water surface and reflected the light while mackerel eggs never did so.

5.2 Egg staging and identification exercises

The criteria for staging mackerel eggs (Lockwood *et al.*, 1977) and horse mackerel eggs (Pipe and Walker, 1987) have been used by WGMEGS participants since the instigation of the triennial surveys. Following discussions at previous egg-staging workshops (ICES, 2001; 2004; 2007; 2009; 2012; 2015b), and further consultations at this workshop, these egg staging criteria have been reviewed (section 3.2.2). These characteristics are the result of many years of personal experience (from various participants) in staging preserved fish eggs from plankton samples.

During the first round of the egg staging and identifying exercise the main discussion, regarding mackerel eggs, was with the stage 1A and 1B and stage 5. Apparently stage 1B and stage 5 mackerel eggs were not present in the exercise but some participants

did score these stages. To clarify the characteristics that separate these from the other stages several mackerel 1A and 1B and stage 5 eggs were shown on the projector and discussed. Only a few horse mackerel eggs were present in the first roung, the majority of them of stage 1A. In this stage the segmentation of the yolk-sac is clearly visible and makes it easy to distinguish from the other species. Because of this, most participants were well able to distinguish some hake eggs that were present in the trays, because they lack this feature. In the second round, more horse mackerel eggs, also more of the later stages, were present in the trays. This would probably explain the decrease in the overall agreement for the identification of horse mackerel eggs. What also caused problems for participants in both identification rounds, however, was the presence of ling eggs. Their size is well within the lower range of mackerel eggs and they have a large oil droplet as well. It was pointed out, that the eggs of ling are more likely to occur in colder water (< 8.5 °C) and that water temperature could be an indicator for the presence of ling eggs. It would be helpful to get more ling eggs for the next workshop and to collect further information on the correlation between temperature and the presence of ling and mackerel eggs.

Most of the mackerel eggs used for the exercises were validated mackerel eggs that were used in the temperature experiments of the Netherlands. The mackerel eggs were in very good condition while some of the eggs of other species were very old and often in bad condition, which made it easier the recognize mackerel eggs. It was also noticed that mackerel 1A eggs from survey's are most of the time coloured inside while the mackerel 1A eggs from the temperature experiment were transparent.

The first eggs staging and identifying exercise and the discussion afterwards clearly helped to identify mackerel 1A and 1B eggs. The overall agreement for the participants increased from 89% to 100% which looks very promising for the next year survey's. Clearly also the inexperienced participants improved their skills.

To be able to properly train the participants during these workshops it is imperative to have samples of good quality eggs of the main species: mackerel, horse mackerel, hake, megrim and ling. All participants of the WGMEGGS 2019 are therefore requested to collect eggs of these species, preferable validated from artificial fertilization experiments. It would also be good if information on the origin of the eggs would be given to aid the identification. For samples of the surveys, these data are always available, providing assurance for the quality of the identification.

5.3 Fecundity, atresia and POF staging (ToR e)

5.3.1 Ovary screening and analysis

Apart from improving the definitions of certain phases of the ovary (see section 4.4.1) and detailing histomorphological aspects of oocyte stages, i.e. what is transferred to templates, it was agreed that taking a section of the ovary instead of a spoonful would improve screening as updated in the manual (SISP.5). On the one hand, POF staging will be improved which is essential to the estimation of spawning frequency and on the other hand, the presence of spawning markers will be more likely to detect, which will improve potential fecundity estimates.

5.3.2 POF staging criteria and analysis

The WKFATHOM came up with a criteria to stage POFs, but because there are too many exceptions to the criteria, it continues being difficult for the participants to agree. However, few key features were agreed to define the transition of POF stages, which

in some cases is critical because these consecutive stages belong to different daily cohorts.

5.3.3 Alpha atresia criteria and intensity image analysis

The decision to classify an oocyte as late alpha atresia if the chorion layer has any break more than twice its width helped the readers to identify early alpha atresia profiles. The results of the atresia exercise have reminded the participants that profile touching the forbidden line are not counted but points do. Besides, when the ovary membrane is open, only those negative points that would have fallen out if the membrane was closed should be counted. As these are frequent omissions, it was agreed that it is a good practice to do it together with more than one person.

5.3.4 Potential fecundity image analysis

Due to the variance observed, it was recommended that when manual counting, readers must stick to the manual, i.e. the oocyte should completely fill the circle in ImageJ to be included in the manual counting; if there is a hole must be leave it.

5.3.5 Micropippette sampling analysis

The experience in the laboratory showed that it is essential to have fecundity sampling material in an adequate state of maintenance. The plunger seems to be sensitive to the regular use and requires to be checked if loose or not, so that there are no problems at the time of sampling and the sample is collected properly.

6 Other items discussed at the workshop

6.1 MEGS on board procedures for collecting mackerel and horse mackerel eggs

Each survey participant presented their procedures for collecting and analysing plankton samples on board their vessels. The procedures are summarized in the following section and give a good overview of the different ship board applications of each participating country.

Scotland

- Scottish version of a Gulf VII sampler, SCANMAR sensor for depth, temperature and salinity, internal and external flowmeter
- In-house RADOS system for monitoring depth during haul and trawl haul data
- After 3 hours fixation picking of eggs
- Second pick after 36 hours fixation
- Third pick if necessary
- Species staged, MAC, HOM, HAK, Lin identified, rest others
- No subsampling, all eggs counted, no need to subsample because samples are small
- Leica MZ6 stereomicroscope, calibrated eye piece graticule

Germany

- Hydrobios Nackthai sampler with Hydrobios CTD, internal and external flowmeter
- Samples fixed for 12 hours in formaldehyde
- Sample sprayed
- Spraying repeated 2 to 3 times
- Eggs sorted and identified and staged under microscope
- Check samples for remaining eggs in the lab after the survey
- Subsampling is done, mix sample and take a pipette sample, all eggs are counted

Ireland

- Gulf VII sampler with Hydrobios CTD, internal and external flowmeter
- Normally have a live feed from the CTD, except when on commercial vessel, than SCANMAR is used.
- Crew wash the net down and bring codend to the lab
- First pick after 1 to 3 hours
- Second pick after 36 hours
- No subsampling, all eggs counted
- Summer, use spray technique
- All samples sorted and eggs identified and staged on board, might not be the case on commercial vessel

The Netherlands

- Gulf VII sampler with Seabird CTD, with internal and external flowmeter
- Live feed of depth, temp and salinity
- Crew wash down the net and bring sample to the lab. Camera is available to check the washing of the net by the crew
- After 24 hours fixation sort eggs using spray technique

- Spray few times until few eggs remain, after that check manually
- Image analyses to identify and stage the eggs, using ObjectJ
- Subsampling when necessary using a Folsom splitter

Spain, IEO

- Bongo40 sampler, internal flowmeter
- Seabird 25 or 37 attached. CTD measurements separately from the Bongo sample
- In March also use CUFES specifically for sardine eggs
- One sample in formaldehyde, one in ethanol
- Formaldehyde sample eggs are identified and staged on board, check samples in the lab after return from the survey
- All eggs are counted, and for staging eggs are subsampled.

Spain, AZTI

- Bongo40 with an RBR-CTD and manual flowmeter.
- SCANMAR for live-feed of depth of the net
- Once a day CTD data are down-loaded
- Sample fixed in formaldehyde
- At end of the transect check the sample for eggs
- After 48 hours sort eggs
- Spray method used for large samples, otherwise manual sorting
- Identification under stereomicroscope
- All eggs are counted, for staging samples are subsampled using a Folsom splitter

Faroese

- Have been using a Bongo with Seabird CTD. For 2019 survey will use a Gulf VII sampler with Seabird CTD and internal and external flowmeters
- Two shifts, one will sort and other will identify
- Subsampling will be used

Portugal

- CalVET40, 150 um
- Also CUFES and Bongo60 (200 and 500 um)
- CTDF and thermo-salinometer
- Vertical tows 3 to 5 m from the bottom
- If eggs appear in CUFES a CalVET sample is carried out. If no eggs appear in CUFES two stations in a row, the transect is ended.
- One sample in formaldehyde, other in ethanol
- Sorting, identification and staging done in the laboratory after the survey
- Staging in 11 stages
- Ethanol samples used for genetic identification of fish eggs

Norway

- Gulf VII sampler with Hydrobios CTD, internal and external flowmeters
- Formaldehyde fixed eggs
- Use image analyses for identification and staging

Denmark

- In the Baltic for their cod egg survey use the Bongo60 with 500 and 350um mesh with a baby-bongo with 150um mesh

7 References

Arkhipov A.G. and Mamedov, A. A. 2008. Ichthyoplankton of the Azores Seamounts. Journal of Ichthyology, Vol 8(3): 259-267.

Armstrong, M.J., Connolly, P., Nash, R.D.M., Pawson, M., Alesworth, E., Coulahan, P.J., Dickey-Collas, Milligan, S.P., O'Neill, M., Witthames, P.R. and Woolner, L. 2001. An application of the annual egg production method to estimate the spawning biomass of cod (*Gadus morhua* L.), plaice (*Pleuronectes platessa* L.) and sole (*Solea solea* L.) in the Irish Sea. ICES Journal of Marine Science, 58: 183 - 203.

Coombs, S.H. 1994. Identification of eggs of hake, *Merluccius merluccius.* J. Mar. biol. Ass.U.K. (1994), 74, 449-450.

Coombs, S.H. and Mitchell, C.E. 1982. The development rate of eggs and larvae of the hake, *Merluccius merluccius* (L.) and their distribution to the west of the British Isles. J. Cons. Int. Explor. Mer, 40: 119-126.

Cunha, M.E., Vendrell, C. and Gonçalves, P. 2007. Experimental study of the dependence of embryonic development of *Trachurus trachurus* eggs on temperature. ICES Journal of Marine Science, 65: 17 – 24.

D'Ancona *et al.* 1956. Fauna e Flora del Golfo di Napoli, Monographia 38: Uova, larve e stadi giovanili di Teleosti, Pubblicata dalla Stazione Zoologica di Napoli, 4 vols.

Ehrenbaum, E. 1905-1909. Eier und Larven von Fischen. *Nordisches Plankton*, **1**, 413pp. Lipsius & Tischer, Leipzig.

Eltink, G. 2007. The spray technique: a new method for an efficient separation of fish eggs from plankton. Journal of Plankton Research, **29**, 871-880

Fahay, M. P. 1983. Guide to the early stages of marine fishes occurring in the Western North Atlantic Ocean, Cape Hatteras to the Southern Scotian Shelf, Journal of Northwest Atlantic Fisheries Science, **4**, 423pp.

Froese, R. and Pauly, D. (Eds.) 2003. FishBase, WWW Pulication, www.fishbase.org , version 12 October 2003

Fonn, M., Thorsen, A., Alvarez, P., Pérez, J.R., Nunes, C., Garabana-Barro, D., Korta, M., Pennock, I., O'Hea, B. and Damme, C.J.G. van, 2015. WGMEGS Manual for the AEPM and DEPM estimation of fecundity in mackerel and horse mackerel. Version 11. SISP 5-WGMEGS-AEPM & DEPM. 83pp.

Gaetani, D. 1937. Uova, sviluppo embrionale e stadi post-embrionali negli *Sparidi* 5. *Box boops* L. Memoria R. Comitato Talassografico Italiano 241: 1 – 14.

Gonçalves, P., Henriques, E. and Angélico, M.M. 2012. Co-occurence of *T. trachurus* and *T. picturatus* spawners in Atlantic Iberian waters. Can the eggs of the two species be distinguished in plankton samples? Fisheries Research – Special Number. Fish Reproduction. *In Press*

Holt, E. W. L. 1893. Survey of fishing grounds, west coast of Ireland, 1890-91: on the eggs and larval and post-larval stages of teleosteans, Scientific Transactions of the Royal Dublin Society, Ser. 2, **5**, 121pp.

Holt, E. W. L. 1898. Notes on the reproduction of teleostean fishes in the south-western district, J.M.B.A., **5:**107-155.

ICES, 2001. Mackerel and Horse Mackerel Egg Staging and Histology Workshop. ICES CM 2001/G:01.

ICES, 2004. Workshop on Mackerel and Horse Mackerel Egg Staging and Identification. ICES CM 2004/G:13.

ICES, 2005. Report of the Working Group on Mackerel and Horse Mackerel Egg Surveys. ICES CM 2005/G:09.

ICES, 2006. Report of the Working Group on Mackerel and Horse Mackerel Egg Surveys (WGMEGS). ICES CM 2006/LRC:09.

ICES, 2006. Workshop on Mackerel and Horse Mackerel Egg Staging and Identification. ICES CM 2006/LRC:17.

ICES, 2009. Workshop on Mackerel and Horse Mackerel Egg Staging and Identification. ICES CM 2009/LRC:13.

ICES, 2012. Report of the Workshop on Egg staging, Fecundity and Atresia in Horse mackerel and Mackerel (WKFATHOM). ICES CM 2012/SSGESST:17.

ICES, 2015a. First Interim Report of the Working Group on Mackerel and Horse Mackerel Egg Surveys (WGMEGS). ICES CM 2015/SSGIEOM:09.

ICES, 2015b. Report of the Workshop on Egg staging, Fecundity and Atresia in Horse mackerel and Mackerel (WKFATHOM). ICES CM 2015/SSGIEOM:01

ICES, 2018. Report of the Working Group on Mackerel and Horse Mackerel Egg Surveys. ICES CM 2018/EOSG:17.

ICES 2019. Manual for the Mackerel and Horse Mackerel Egg Surveys (MEG): Sampling at Sea. Version 2.2. ICES SISP 6. In press

Johnstone, J., Scott, A. and Chadwick, H. C. 1934. The Marine Plankton, Hodder and Stoughton Limited, London, 194p.

King, D. P. F., O'Toole, M. J., and Robertson, A. A. 1977. Early development of the South African maasbanker *Trachurus trachurus* at controlled temperatures. Fisheries Bulletins of South Africa. 9: 16 – 22.

Kloppmann, M., Milligan, S., Ulleweit, J., Burns, F., Angélico, M.M., O'Hea, B. and Damme, C.J.G. van, 2015. Manual for the mackerel and horse mackerel egg surveys (MEGS): sampling at sea. Version 2. SISP 6 - MEGS V2. 99pp.

Kramer, D. 1960. Development of eggs and larvae of the Pacific Mackerel and distribution and abundance of larvae 1952-56. Fish. Bull. U.S., 60: 393-438.

Lasker, R. 1985. An egg production method for estimating spawning biomass of pelagic fish: Application to the northern anchovy, *Engraulis mordax*. 36. NOAA Technical report NMFS 36: 110p.

Lockwood, S.J., Nichols, J.H. and Coombs, S.H. 1977. The development rates of mackerel (*Scomber scombrus* L.) eggs over a range of temperature. ICES CM 1977/J:13, 8pp.

Lockwood, S.J., Nichols, J.H. and Dawson, W.A. 1981. The estimation of a Mackerel (*Scomber scombrus* L.) spawning stock size by plankton survey. J. Plankton Research, No 3 (2): 217-233p.

Marques V., Chaves C., Morais A., Cardador F., Stratoudakis Y. 2005. Distribution and abundance of snipefish (*Macroramphosus* spp.) of Portugal (1998–2003). Sci. Mar. 69:563–576.

Olivar, M-P and Fortuño, J-M. 1991. Guide to Ichthyoplankton of the Southeast Atlantic (Benquela Current Region). SCI. MAR., 55(1): 1-383.

Padoa, E. 1956. *in* Lo Bianco, S. Uova, larvae e stadi giovanili di Teleoste – Divisione: Carangiformes, Fauna e flora del golfo di Napoli, Stazione Zoologica di Napoli, Monografia 38 and 3ª Puntata, 2ª parte: 548 – 576.

Pipe, R.K. and Walker, P. 1987. The effect of temperature on the development and hatching of scad (*Trachurus trachurus*, L.) eggs. J. Fish Biol., 31: 675-682p.

Porebski, J. 1975. Application of the surface adhesion test to identify the eggs of the hake *Merluccius spp*. Colln. Scient Papers: Int Commission for SthEast Atlantic Fisheries 1975. Vol 2 102-106p.

Rasband, W. S. 1997-2008. ImageJ. U.S. National Institutes of Health, Bethesda, Maryland, USA.

Russell, F.S. 1976. The eggs and planktonic stages of British marine fishes. Academic Press Inc. (London) Ltd., 524 p.

Shaw, M. D. 2003. *Personal communication.*

Spartà, A. 1936. Contributo alla conoscenza di uova, stadi embrionali e post-embrionali in *Macroramphosus scolopax* L. R. Com. Talassog. Ital. Mem. 225: 14 pp.

Annex 1: List of participants

Annex 1a. List of participants for the WKFATHOM-egg identification meeting in Bremerhaven, Germany, 8–12 October 2018.

Name	Address	Phone/Fax	E-mail
Anders Thorsen	Institute of Marine Research Nordnes P.O. Box 1870 5817 Bergen Norway	+47 95873368	anders.thorsen@hi.no
Bahar Mozfar	Institute of Marine Research Nordnes P.O. Box 1870 5817 Bergen Norway		bahar.mozfar@hi.no
Beatriz Beldarrain	AZTI, Herrera Kaia Portualdea z/g 20110 Pasaia (Gipuzkoa) Spain		bbeldarrain@azti.es
Birgit Suer	Thünen Institute Institute of Sea Fisheries Herwigstr. 31 27582 Bremerhaven Germany		birgit.suer@thuenen.de
Brendan O' Hea	Marine Institute, Rinville, Oranmore, Galway, Ireland.	+35391 387304	brendan.ohea@marine.ie
Cindy van Damme (chair)	IMARES, Haringkade 1, 1976 CP IJmuiden, The Netherlands	+31 (0) 317 487078	cindy.vandamme@wur.nl
Hannah Lloyd-Hartley	CEFAS Lowestoft Laboratory, Pakefield Road, Lowestoft, Suffolk, NR33 0HT. United Kingdom.		hannah.LloydHartley@cefas.co.uk
Ewout Blom	IMARES, Haringkade 1, 1976 CP IJmuiden, The Netherlands		ewout.blom@wur.nl
Finlay Burns	Marine Scotland Science Marine Laboratory 375 Victoria Road Aberdeen AB11 9DB United Kingdom	+44 1 224 29 5376	F.Burns@MARLAB.AC.UK
Sólva Káradóttir Eliasen	Faroe Marine Research Institute Nóatún 1 P.O. Box 3051 110 Tórshavn Faroe Islands	+298 35 39 00	solvae@hav.fo

Durita Sørensen	Faroe Marine Research Institute Nóatún 1 P.O. Box 3051 110 Tórshavn Faroe Islands	+298 35 39 00	duritas@hav.fo
Bastian Huwer	DTU Aqua - National Institute of Aquatic Resources Kemitorvet Building 202, Room 4134 2800 Kgs. Lyngby Denkmark	+45 35883467	bhu@aqua.dtu.dk
Jens Ulleweit	Thünen Institute Institute of Sea Fisheries Herwigstr. 31 27582 Bremerhaven Germany	+49 471 94460 117	jens.ulleweit@thuenen.de
Jim Drewery	Marine Scotland Science Marine Laboratory 375 Victoria Road Aberdeen AB11 9DB United Kingdom	+44 1224 295354	J.Drewery@marlab.ac.uk
Maria Krüger-Johnsen	DTU Aqua -National Institute of Aquatic Resources Kemitorvet Building 202, Room 4134 2800 Kgs. Lyngby Denmark		mkru@aqua.dtu.dk
Gersom Costas	Instituto Español de Oceanografía Centro Oceanográfico de Vigo Subida a Radio Faro 50 Cabo Estai – Canido 36390 Vigo (Pontevedra) Spain		gersom.costas@ieo.es
Luisa Iglesias	Instituto Español de Oceanografía Centro Oceanográfico de Vigo Subida a Radio Faro 50 Cabo Estai – Canido 36390 Vigo (Pontevedra) Spain		luisa.iglesias@ieo.es

Matthias Kloppmann **chair**	Thünen Institute Institute of Sea Fisheries Herwigstr. 31 27582 Bremerhaven Germany	+49 471 94460 367	matthias.kloppmann@thuenen.de
Merete Fonn	Institute of Marine Research Nordnes P.O. Box 1870 5817 Bergen Norway	+47 41304063	merete.fonn@hi.no
Paula Alvarez	AZTI, Herrera Kaia Portualdea z/g 20110 Pasaia (Gipuzkoa) Spain	0034 946574000	palvarez@azti.es
Isabel Riveiro	Instituto Español de Oceanografía Centro Oceanográfico de Vigo Subida a Radio Faro 50 Cabo Estai – Canido 36390 Vigo (Pontevedra) Spain		isabel.riveiro@ieo.es
Sakis Kroupis	Thünen Institute Institute of Sea Fisheries Herwigstr. 31 27582 Bremerhaven Germany		sakis.kroupis@thuenen.de
Thassya dos Santos Schmidt	Institute of Marine Research Nordnes P.O. Box 1870 5817 Bergen Norway		thassya.dos.santos.schmidt@hi.no
Timo Meißner	Thünen Institute Institute of Sea Fisheries Herwigstr. 31 27582 Bremerhaven Germany		timo.meissner@thuenen.de

From left to right: Durita, Gersom, Luisa, Isabel, Paula, Beatriz, Jim, Hannah, Merete, Jens, Sakis, Jim, Maria, Ewout, Thassya, Bahar, Elisabethe, Anders, Matthias, Brendan, Sólva, Birgit

Annex 1b. List of participants for the WKFATHOM-fecundity estimation meeting in Bergen, Norway, 19–23 November 2018.

Name	Address	Phone/Fax	E-mail
Anders Thorsen	Institute of Marine Research Nordnes P.O. Box 1870 5817 Bergen Norway		anders.thorsen@imr.no
Antonio Solla Covelo	Instituto Español de Oceanografía Centro Oceano-gráfico de Vigo Subida a Radio Faro 50 Cabo Es-tai – Canido 36390 Vigo (Pon-tevedra) Spain	+34 986 492 111 +34 986 498 626	antonio.solla@ieo.es
Brendan O'Hea	Marine Institute, Rinville, Oranmore, Galway, Ireland.	+35391 387304	brendan.ohea@marine.ie
Cindy van Damme (chair)	IMARES, Haringkade 1, 1976 CP IJmuiden, The Netherlands	+31 (0) 317 487078	cindy.vandamme@wur.nl
Cristina Nunes	Portuguese Institute for the Sea and the Atmosphere (IPMA) Avenida de Brasilia 1449-006 Lisbon Portugal	+351 213027000	cnunes@ipma.pt

Dolores Garabana Barro	Instituto Español de Oceanografía Centro Oceanográfico de A Coruña Muelle de las Animas s/n P.O. Box 130 15001 A Coruña Spain		dolores.garabana@ieo.es
Grethe Thorsheim	Institute of Marine Research Nordnes P.O. Box 1870 5817 Bergen Norway		grethe.thorsheim@imr.no
Hanz Wiegerinck	IMARES, Haringkade 1, 1976 CP IJmuiden, The Netherlands		hanz.wiegerinck@wur.nl
Ineke Pennock	IMARES, Haringkade 1, 1976 CP IJmuiden, The Netherlands		ineke.pennock@wur.nl
Inger Hornum	DTU Aqua - National Institute of Aquatic Resources Jægersborg Allé 1 2920 Charlottenlund Denmark	+45 35 88 34 17	ih@aqua.dtu.dk
Maria Korta	AZTI, Herrera Kaia Portualdea z/g 20110 Pasaia (Gipuzkoa) Spain	0034 946574000	mkorta@azti.es
Merete Fonn	Institute of Marine Research Nordnes P.O. Box 1870 5817 Bergen Norway		merete.fonn@imr.no
Hannah Holah	Marine Scotland Science Marine Laboratory 375 Victoria Road PO Box 101 Aberdeen Scotland AB11 9DB United Kingdom		Hannah.Holah@gov.scot
Paula Alvarez	AZTI, Herrera Kaia Portualdea z/g 20110 Pasaia (Gipuzkoa) Spain	0034 946574000	palvarez@azti.es
Gersom Costas	Instituto Español de Oceanografía	+34 986 492 111 +34 986 498 626	gersom.costas@ieo.es.

	Centro Oceano-gráfico de Vigo Subida a Radio Faro 50 Cabo Es-tai – Canido 36390 Vigo (Pon-tevedra) Spain		
Jonna Tomkiewicz	DTU Aqua - Na-tional Institute of Aquatic Resources Jægersborg Allé 1 2920 Charlotten-lund Denmark		jt@aqua.dtu.dk
Ewout Blom	IMARES, Haringkade 1, 1976 CP IJmuiden, The Netherlands		ewout.blom@wur.nl
Thassya Christina dos Santos Schmidt	Institute of Marine Research Nordnes P.O. Box 1870 5817 Bergen Norway		thassya.dos.san-tos.schmidt@hi.no

WKFATHOM_Fecun dity (2018)

Top to bottom: Cristina, Inger, Ineke, Ewout, Hanz, Antonio, Brendan, Gersom, Jonna, Grethe, Maria, Hannah, Thassya, Lola, Cindy, Anders, Merete and Paula.

Annex 2: Agenda

Annex 2a. Agenda for the WKFATHOM-egg identification meeting in Bremerhaven, Germany, 8 –12 October 2018.

Monday 8th October

10:00 Start of the meeting, practical stuff etc.

10:15 Introduction round, practicalities, division of tasks, etc.

10:30 Presentation: The mackerel and horse mackerel egg survey (MEGS): Identification and staging of fish eggs – **Matthias Kloppmann**

11:00 Presentations: The participating institutes – short presentation by representatives of the participating institutes on their egg surveys

12:30 Lunch

13:30 Identification and staging of a couple of fish eggs in plenary

14:30

- 1st round of egg identification and staging (ToR b)
- 1st round Spray Method (ToR a)

17:30 End of the day

Tuesday 9th October

09:00

- Continue 1st round of egg identification and staging (ToR b)
- Continue Spray method (ToR a)

12:30 Lunch

13:30

- Finish 1st round Spray method (ToR a)
- Finish 1st round of egg identification and staging (ToR b)

14:30

- Planning for the 2019 survey – **Brendan O'Hea**
- Update survey manual and standard protocols – **Jens Ulleweit** (ToR d)

17:30 End of the day

Wednesday 10th October

09:00 Discussion of results of 1st rounds of Spray Method and of egg identification and staging (ToR b)

12:30 Lunch

13:30

- 2nd round of egg identification and staging (ToR b)
- 2nd round of Spray method (ToR a)

16:30 End of the day

17:30 Visit of the Historic Museum of Bremerhaven

19:00 Dinner at the "Seute Deern" Restaurant

Thursday 11th October

09:00

- Continue 2nd round of egg identification and staging (ToR b)
- 2nd round Spray method (ToR a)
- Update pictures and descriptions and review available information on species identification and egg staging (ToR c and d)
- Write report

12:30 Lunch

Thursday 11th October (continued)

13:30

- 2nd round Spray method (if necessary, ToR a)
- Finalize standard pictures and descriptions set for both species (ToR c and d)
- Update survey manual and standard protocols – **Jens Ulleweit** (ToR d)
- Write report, recommendations

17:30 End of the day

Friday 12th October

09:00

- Discussion of results of 2nd round of egg identification and staging (ToR b)
- Discussion of results of the spray method
- Finalize survey manual and standard protocols (ToR d)
- Finalize report, recommendations etc

12:30 End of the meeting

Annex 2b. Agenda for the WKFATHOM-fecundity estimation meeting in Bergen, Norway, 19–23 November 2018.

Monday 19 November: Introduction and state of the art

10:00	Start of the meeting, domestics (WUR)
10:15	Introduction round.
10:25	WKFATHOM: Objectives and agenda (working plan for the week) (AZTI)
10:35	Presentations: State of the art • WGMEGGS: adult sampling design (to be decided) • AEPM/DEPM: adult sampling on board and procedures (All Institutes) • Samples labelling and packaging (IMR) • Overview: Templates, Parameters, Rings-tests (WUR)
11:30	Lab practice: Introduction to image analysis (IMR) Lab practice 1 • Introduction to fixation issue (WUR) • Histological slides reading from pictures.
12:30 – 13.30	Lunch break
13:30 – 17:00	Lab practice 2 • Introduction to screening analysis (IMR) • Demonstration of NDP.View2 • Screening analysis from pictures Discussion: Results and % of agreement. Improvement.
17:00	Plenary.
17:30	End of the day.

Tuesday 20 November: DEPM

9:00 -12:00	Presentations DEPM: adult sampling, sample procedure and analysis (IEO) DEPM: adult sampling, sample procedure and analysis (IPMA) WGALES: Spawning fraction (IMR/WUR) Plenary
12:00	Lab practice 3 • Introduction to POF stages identification (IEO) • POF staging from histological slides
12:30 – 13.30	Lunch break
13:30 – 17:00	Lab practice 3 • POF staging from histological slides (if not finished). • Discussion: results and % of agreement. Improvement. • Modifications in the manual.
17:00	Plenary
17:30	End of the day.

Wednesday 21 November: AEPM

9:00 -12:00	Presentations
	AEPM: adult sampling, sample procedures and analysis (AZTI)
	AEPM: fecundity (IEO)
	AEPM/DEPM: Comparative spoon vs ovary section for screening (IEO)
	Plenary
12:00	Lab practice 4 • Introduction to atresia (IMR) • Atresia estimation from images.
	Micropipettes test
12:30 – 13.30	Lunch break
13:30 – 17:00	Lab practice 4 • Atresia images from scanner images (if not finished). • Discussion: results and % of agreement. Improvement. • Modifications in the manual.
17:00	Plenary
17:30	End of the day.
19:00	Dinner

Thursday 22 November: Related issues

9:00 -12:00	Lab practice 5 • Introduction to fecundity analysis (IMR) • Fecundity analysis from pictures • Discussion: Results and % of agreement. Improvement.
12:00	Presentations
	Year of the mackerel (WUR)
	CLIMRATES (IMR)
12:30 – 13.30	Lunch break
13:30 – 17:00	Subgroups • Manual AEPM/DEPM • Report
17:00	Plenary
17:30	End of the day.

Friday 23 November: Report and close

9:00 -12:30	Plenary
	Report
	Manual
	ToRs and recommendations
12:30	End of the workshop.

Annex 3: Recommendations

RECOMMENDATION	ADRESSED TO
1. It is recommended that all WGMEGS participants carry out artificial fertilizations of any species, which have eggs similar to those of mackerel and horse mackerel. It would be useful if egg and oil globule diameters are measured and that photographs are taken of as many stages as possible. It would also be beneficial if the eggs were preserved at various stages of development and any morphological changes noted following fixation. These eggs should be made available for analysis during the next workshop (scheduled for 2021).	WGMEGS
2. All survey participants are requested to measure formaldehyde preserved egg diameters and oil droplet diameters of 100 hake, 100 mackerel and 100 horse mackerel eggs during each individual cruise, to identify changes in egg diameter over spawning time and area. Also the development stage should be reported. Hake measurements should only be included from SAT removed eggs.	WGMEGS
3. All survey participants are requested to investigate genetic or other molecular (MALDI-TOF) analyses of fish eggs to aid species identification.	WGMEGS, WGALES
4. WKFATHOM recommends that institutes provide continuity of staff to carry out the plankton identification and staging to ensure a high quality standard of the survey. It is the institutes responsibility to provide appropriate training for new staff in advance of the survey. This should be done through institute workshops, as one week of training during WKFATHOM is not enough to turn trainees into experts.	WGMEGS, WGALES
5. WKFATHOM encourages exchanges of staff between participating institutes, to allow exchange of knowledge and increase expertise amongst survey participants.	WGMEGS
6. All survey participants should take pictures of mackerel, horse mackerel and also species with similarly sized eggs in the different development stages of formaldehyde fixed eggs.	WGMEGS
7. T-S data from the MEGS should be included into the egg and larvae database	ICES data centre, DIG